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Cultivation of the Murine SK Strain of Poliomyelitis Virus in Developing Eggs.*

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Dunham and Parker,[1] Gard,[2] and recently Riordan and Sá-Fleitas[3] have reported the cultivation of Theiler's mouse encephalomyelitis virus in developing eggs. Similar attempts to propagate strains of human poliomyelitis virus in eggs have failed;[4-7] a doubtful result, in which one monkey inoculated with egg passage material developed paralysis long after the usual incubation period, has been reported by Gard.[8]

The present report deals with the propagation in fertile eggs of the murine SK strain of poliomyelitis virus described by Jungeblut and Sanders[9] as a strain they had adapted to mice from a monkey passage virus, originally isolated by Trask, Vignec, and Paul[10] from the stools of a child with nonparalytic poliomyelitis. Unlike the mouse-adapted Lansing strain,[11] this strain no longer readily induces typical poliomyelitis in monkeys.[12] It is distinctive also in that it attains high concentrations in mice and is infectious for them by a variety of routes.

Sanders and Jungeblut[13] in 1942 reported the cultivation of this strain in serum ultrafiltrate containing embryonic mouse tissue and made observations on the conditions which favor its growth. The amount of growth was influenced by the relative amount of nervous tissue represented in the cultures and was greatest in the presence of brain tissue. No growth was obtained when embryonic chick tissue was used in place of mouse tissue, and none was obtained in embryonated eggs. Schultz and Irwin[14] were able to confirm the multiplication of this strain in the presence of minced embryo mouse brain tissue and serum ultrafiltrate. The virus was carried through 14 culture passages. It was also carried through a similar number of passages in which minced mouse embryo intestines was used in place of brain tissue. The mouse-adapted Lansing strain of poliomyelitis virus failed to show growth under similar conditions. Chick embryo tissue was not tried.

The present observations were made on fertile eggs incubated for 9 days at 100°F in an egg incubator. The initial inoculum consisted of 0.1 ml of a 10^{-5} dilution of filtered mouse brain suspension, prepared as follows: A 10% mouse brain suspension in broth was filtered through a coarse Mandler candle and diluted serially, in 10-fold dilutions with broth, through to a final dilution of 10^{-7}, counting the unfiltered dilution as 10^{-1}. The 10^{-5} dilution induced infection in 3 out of 3 mice inoculated with 0.025 ml of the dilution intracranially; the 10^{-6} dilution induced infection in 2 out of 4 mice, while 10^{-7} dilution failed to induce infection in all of 5 mice so inoculated.

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TABLE I.
Incidence of Deaths in Embryos and Results of Tissue Assays for Virus in Individual Egg Passages.

Passage No.	Route of inoculation	Incidence of deaths in embryos	Tissues assayed	M.I.D.¶ of virus per g tissue
3	C-A*	11/12§		
	Y-S†		Heads	4×10^5
	I-E‡			
			C-A	4×10^5
5	C-A	5/5	Heads	4×10^5
			Viscera	4×10^6
			C-A	4×10^4
	Y-S	4/5	Heads	4×10^6
			Viscera	4×10^7
	I-E	3/4	Brain	4×10^5
6	C-A	5/6	Heads	4×10^6
	Y-S	5/6	Viscera	4×10^7
7	C-A	5/6	Heads	4×10^6
	Y-S	3/4	Viscera	4×10^6
8	C-A	5/6	Heads	4×10^6
	Y-S	6/6	Viscera	4×10^6
10‖	C-A	2/6	Heads	4×10^6
	Y-S	3/6	Viscera	4×10^6
11	C-A	5/6	Heads	4×10^7
12	C-A	11/18	"	4×10^6
13‖	C-A	5/13	"	4×10^5
14	C-A	14/19	"	4×10^5

* Chorioallantoic route.
† Yolk-sac route.
‡ Intra-embryonic route.
§ Denominator = total number of eggs inoculated; numerator, number of deaths in embryos between the 2nd and 5th days.
‖ Inoculated eggs incubated at 37°C, instead of 35°C.
¶ M.I.D. = minimal infectious doses.

During the first 5 passages, groups of eggs were inoculated by one of 3 different routes: (a) chorioallantois according to Burnet,[15] (b) into the yolk sac according to Cox,[16] and (c) intraembryonically according to Elmendorf and Smith.[17] After inoculation, the eggs were incubated at 35°C and were candled daily over a period of 5 days to determine the viability of the embryos. A high per cent became nonviable. A few which became nonviable within 24 hours were discarded. Eggs in which death of the embryo occurred between the 2nd and 5th day were stored in a refrigerator at 4°C. On the 5th day all of the embryos were harvested, including those still viable; the material was pooled and prepared for passage to the next group of eggs. In preparing the material for the next passage, the heads and necks of the individual embryos were pooled and ground in a mortar to a uniform paste. This was emulsified in broth to make a 10% suspension. After centrifugation at 2000 r.p.m. for 10 minutes the supernatant was filtered through a coarse Mandler candle. One-tenth ml of this filtrate served as the inoculum for the next passage.

[15] Burnet, F. M., Medical Research Council, Special Reports Series No. 220, 1936, pp. 1-58.

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[17] Elmendorf, J. E., and Smith, H. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 171.

Titrations were carried out on filtrates from each passage.

In assaying the virus content of the embryos from the 5th passage, eggs were separated on the basis of the routes by which the inoculations were made and different portions of the embryos in each group were titrated separately for virus content. These portions included pools of the chorioallantois, pools of the heads of embryos, and pools of the abdominal viscera (kidneys, liver, spleen, stomach and intestine). In those inoculated by the intraembryonic method, pooled brains only were titrated for virus.

After the 5th passage the intraembryonic method was abandoned, and the passages made by the 2 remaining routes were carried independently of each other. In the passages which were made by the chorioallantoic route, the material for the next inoculation was always obtained from the pooled heads of embryos harvested from the previous passage; in those made by the yolk sac route, the material inoculated was always harvested from the pooled viscera of embryos previously infected by the yolk sac route. The purpose of these 2 separate lines of passage was to determine whether the relative difference in the amount of nervous tissue represented would influence appreciably the amount of virus produced.

It has been stated that a high per cent (about 80%) of the embryos died between the 2nd and 5th days. These deaths occurred from the first passage onward. No distinctive gross or microscopic lesions in either the chorioallantois or the embryos have thus far been identified, but this is under further study. The incidence of deaths in embryos in eggs incubated at 37°C was lower (40%) than that in eggs incubated at 35°C.

Our observations are summarized in Table I. Results on certain of the passages have been omitted to save space. These are in agreement with those given. All of the titrations were carried out by inoculating the individual dilutions of the passage material intracranially into groups of 3 to 5 white mice, each with 0.025 ml of the dilution, and 50% infection in the individual groups was counted as the end point.

Neutralization tests against anti-SK serum, prepared before these studies were initiated, were carried out with virus from the 10th egg passage. The filtrate used in the tests titered 10^{-5}, based on 50% infection. It was employed in a dilution of 10^{-4} and 0.5 ml of this dilution was treated with an equal volume of the individual serum dilutions. Complete neutralization was obtained in serum dilutions up to and including 1:512, the highest serum dilution tested. Theiler's GD-VII strain of mouse encephalomyelitis virus was not neutralized by the serum.

Summary. The murine SK strain of poliomyelitis virus was carried through 14 passages on developing eggs without evident diminution of virus content. Its multiplication was associated with a high incidence of deaths in the embryos. The incidence of deaths was influenced by the temperature at which the eggs were incubated after inoculation.†

† Since this manuscript was submitted, we have made observations which indicate that the Lansing strain also, may be cultivated under these conditions.

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Alopecia in Rats Fed Certain Soybean Oil Meal Rations.*

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It is generally believed that the rat does not require dietary sources of inositol and biotin when purified rations are fed. However, certain dietary constituents may in some

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