tiveness in blocking conversion of desmosterol to cholesterol is not clear. However, studies by Palopoli(18), as well as evidence presented here, indicate that activity is limited to certain compounds and is neither shared by all those containing the diethylaminoethoxy group *per se,* nor limited to any other single moiety in the molecule. Testing of additional substances for inhibitory effects on cholesterol synthesis might bring about a better understanding of enzymatic processes involved and help to establish the most favorable chemical structure for maximum inhibitory activity and minimum toxic side effects.

Summary. 1. U-18666A, a new inhibitor of cholesterol biosynthesis, caused marked reduction in serum and liver sterols and appearance of desmosterol in livers of rats. 2. Reduction of serum sterols by U-18666A was obtained in animals in which hypercholesterolemia was induced with Triton WR-1339. 3. U-18666A increased incorporation of acetate-1-C^{14} into liver digitonin precipitable sterols fourfold and into liver fatty acids twofold. 4. Of several other compounds tested for cholesterol inhibitory activity, only hexestrol bis-β-diethylaminoethyl ether was found to cause appearance of desmosterol.

Appreciation is gratefully expressed for technical assistance by Miss Ann E. Butler and Mr. Walter W. Stafford.

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Received October 12, 1962. P.S.E.B.M., 1963, v112.

Cell Cultures for Detection of Trachoma Virus from Experimental Simian Infections.* (28004)

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Two methods have been used to detect the causative agent of trachoma in infected eyes, *i.e.,* microscopic examination of direct smears, and culture of conjunctival specimens in the yolk sac of embryonated eggs(1). Although the latter method is valuable as a research tool, it is not satisfactory as a routine diagnostic procedure. Similar methods have been

* The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

successful for demonstrating inclusion conjunctivitis virus in the eye(2) and the genital tract(3). Recent studies in which egg-established strains of these agents(4) were grown in cell cultures raised the question of using such cultures as a means of isolating the virus directly from the infected eye or genital tract. This report describes comparative tests made with embryonated eggs and with cell cultures for demonstration of trachoma virus in experimental simian infections. Bacteriologic studies were performed to determine the types and relative numbers of organisms in each specimen in an effort to establish the efficacy of the antibiotics employed in viral culture.

Inoculation of monkeys. Strain TRIC/USA-Cal/Cal-1/OT†, isolated from a case of trachoma in California(5), and formerly called strain Bourassa, kindly supplied to us by Dr. Ernest Jawetz, was used in its 16th yolk sac passage to infect 2 adult female rhesus monkeys *(Macaca mulatta).* The right tarsal conjunctiva was rubbed with a cotton applicator soaked in a 20% emulsion, in sucrose-phosphate solution, of infected yolk sac tissue representing the 16th yolk sac passage.

Cell cultures. The McCoy synovial (human) cell line(7), obtained through the kindness of Dr. Morris Pollard, was maintained and used in Eagle's medium plus 10% horse serum (MEM • HoS10), as supplied by Microbiological Associates, Inc. Incubation of stock cultures was at 32° or 35°C. Monolayers were grown on 12 mm circular coverslips in flat-bottomed 16 mm culture tubes containing 0.5 ml MEM • HoS10.

Method of obtaining specimens. Specimens were taken from the conjunctiva in 2.5 ml MEM • HoS10 by 2 methods: (a) washing with the medium by expelling a fraction of one ml into the conjunctival sac and withdrawing it by means of a medicine dropper, and (b) by swabbing the tarsal conjunctiva with a dry cotton swab which was then immersed in the medium. The 2 types of specimens were regularly taken at each examination, in the order named and were usually kept separate for comparative tests. In some cases they were combined, or both taken in the same 2.5 ml of medium. A dry swab was also used to make a direct smear on a slide. At frequent intervals, usually just before specimens were taken, the eye was examined by means of a loupe and its condition recorded.

Inoculation of cell cultures. One ml of each specimen was used for inoculation, in 0.25 ml quantities, of the monolayers in 4 tubes of McCoy cells from which the culture medium had been removed. The tubes were then centrifuged for one hour at 3000 RPM (20°C) in a horizontal centrifuge, following the method of Weiss and Dressler(8). The volume in each tube was then made up to 0.5 ml with MEM • HoS10 containing streptomycin and ristocetin so that final concentrations were 50 μg and 100 μg per ml, respectively. Incubation was at 35°C for 48 hours at which time the coverslips were washed with balanced salt solution, fixed with methyl alcohol, and stained with Lugol's solution. The entire monolayer on each coverslip was scanned for the presence of the typical iodine-staining inclusions, using an 8 mm (20×) objective and 10× oculars. After microscopic examination the coverslips were returned to methyl alcohol for later staining and reexamination, as needed. Many were later stained with periodic acid-Schiff's reagent.

When passages from infected cell cultures were made the cells were suspended in the supernatant medium and treated for 5 minutes in a 250-watt, 10-KC sonic oscillator (Raytheon).

Inoculation of eggs. To a second one ml portion of the specimen an equal volume of MEM • HoS10 was added containing streptomycin and ristocetin to give a final concentration of 4000 μg of each/ml. Four 6-day embryonated eggs each received 0.5 ml of this mixture into the yolk sac. Each egg thus received the same amount of original conjunctival specimen as did each McCoy cell tube. These drugs and dosages for both cell cultures and eggs were chosen on the basis

† This follows the method of designation of isolates recently recommended by an *ad hoc* committee(6).

of previous experiments(9). On days 2 and 5 after inoculation each egg received 1000 μg of each of the 2 antibiotics, given through the air sac(10). Eggs were candled daily for 14 days and yolk sac smears of dead or sacrificed embryos were examined after staining by Macchiavello. Second and occasionally third passages were made with yolk sacs of dead embryos, or with sacs from living embryos harvested on day 8 and day 14.

Bacteriologic studies. Two 0.1 ml amounts of the specimens were each cultured on a fortified blood agar plate(11) by spreading the undiluted fluid evenly with bent glass rods. One plate was incubated aerobically at 37°C and the other under approximately 8% CO_2 tension as attained by candle jar at 37°C. Cultures were assigned numbers at random and were not paired during the investigation. The plates were examined at 24 hours and at 48 hours. Organisms were identified to the generic level by colonial characteristics, cellular morphology, Gram reaction, and, when necessary, by subculture to appropriate media. Colony counts were performed utilizing the Quebec Colony Counter.

Results. The 2 monkeys were examined and conjunctival specimens were obtained at frequent intervals, sometimes daily, after inoculation. Both monkeys developed conjunctivitis within a few days, characterized by the appearance of hyperemia, chemosis, exudate, and follicles. No corneal changes were seen. Because of the planned frequent procurement of specimens an effort was made to minimize the trauma involved in taking material for culture and direct smear. For this reason only washing and swabbing with a cotton applicator were done. A cotton swab for taking the specimen for direct smear was found to be inadequate, at least *after* washing and swabbing for culture, because relatively few cells were found on the slides and in no instance were inclusions identified in direct smears after staining with Lugol's solution.

Monkey H, the first inoculated, with which we developed methods, had a relatively short infection, as judged by ocular signs and by recovery of virus (Table I). Unfortunately, there was some difficulty with the cell line during the critical time of this infection, and the tests for virus were not made with entirely satisfactory cell cultures. Nevertheless, virus was clearly demonstrated on day 7 by inoculation of McCoy cells and possibly on days 4 and 9. The question marks in the 5th column of Table I indicate the observation of a single inclusion body. Although inclusions were not recorded unless they appeared to be typical, we prefer to score as "doubtful" those tests in which only one inclusion was recorded. When 2 or more typical inclusions were observed, the interpretation was "+." With monkey H, virus was recovered in eggs from the one specimen that was positive in cell culture (day 7), from one (day 11) that was clearly negative in cell culture, and from one (day 9) where the cell culture was "doubtful." The infection of monkey M extended over a longer period of time, giving us the opportunity for more extensive studies (Table I).

Most of the conjunctival specimens were processed within 2 hours after they were obtained, but a few of the specimens were frozen in a CO_2 bath and stored at approximately −55°C for periods of 2 to 18 days before being tested. It became clear that this procedure reduced the infectivity of the specimens to a point where they were usually negative by these tests, as indicated in Table I. The effect of freezing is especially striking in the specimens of monkey M on days 42 and 48, each of which was divided into 2 parts. One portion was immediately inoculated into cell cultures and eggs, and the other was frozen, stored, and tested later. In each case virus was demonstrated in the fresh specimen while the frozen and stored portion was negative (Table I).

The studies on monkey M show considerable parallelism between the results with cell cultures and with eggs. The exceptions are: one specimen (day 8, washing) gave a positive test in McCoy cells and a negative test in eggs. In 2 others (swab specimens of days 4 and 8) "doubtful" tests were recorded in cell cultures with a negative test in eggs. In 3 specimens (day 6, washing; 10, swab; 48, combined) the egg test was positive when the cell culture was negative or doubtful. In

TABLE I. Examination of Conjunctival Specimens from Simian Trachoma.

Days after inoculation	Intensity of ocular signs	Type of specimen	Result of culture in McCoy cells: Avg No. inclusions per coverslip	Interpretation	Passage from cell culture to yolk sacs		Results of culture in embryonated eggs: Recovery of virus	No. of passages
(Monkey H)								
2	±	W	0	0			0	2
		S	0	0			0	2
4	±	W & S, stored	.3	?			0	3
7	+	W & S	15	+	C & F	+	+	1
9	++	W & S	.3	?	F	+	+	2
11	+	W & S	0	0			+	2
16	±	W	0	0			0	2
		S	0	0			0	3
(Monkey M)								
2	±	W	0	0			0	2
		S	0	0			0	2
4	+	W	.75	+	C & F	0	+	2
		S	.25	?			0	2
5	++	W, stored	0	0			0	1*
		S, "	0	0			0	1*
6	+++	W	0	0			+	1
		S	0	0			0	2
7	+++	W, stored	0	0			0	2
		S, "	0	0			0	2
8	++	W	.5	+	F	0	0	3
		S	.25	?			0	3
9	++	W, stored	0	0			0	2
		S, "	0	0			0	2
10	±	W	.5	+	F	0	+	1
		S	0	0			+	1
13	±	W	4	+	C & F	0	+	2
		S	4	+	C & F	0	+	1
15	±	W	1	+	C & F	+	+	2
		S	5	+	C & F	+	+	2
16	±	W, stored	.25	?			0	2
		S, "	0	0			0	2
17	+	W	.75	+	F	0	+	1
		S	2	+	F	0	+	1
20	+	W	2.5	+	C & F	+	+	2
		S	15	+	C & F	+	+	1
27	±	W	2	+			+	2
		S	6	+	F	0	+	1
34		W	4.5	+	C & F	0	+	1
		S	1	+	C & F	0	+	1
42	++	W & S, fresh	1	+			+?*	1
		" , stored	0	0			0?*	3
48	+	W & S, fresh	.25	?			+	1
		" , stored	0	0			0	2
55	±	W & S	0	0			Not done	

W = Washing; S = Swab; C = Cells; F = Fluid. ? = Doubtful (one inclusion only observed).
* Fungal contamination complicated further passage, or interpretation of test.

all others the results were the same in both tests.

A comparison of the 2 types of test with respect to both monkeys is presented more concisely in Table II. When all "doubtfuls" are regarded as negative tests, the cell culture method detected virus 17 times, including once when the egg test was negative, and failed to detect virus 5 times when the egg test was positive.

A comparison can also be made between the two methods of taking specimens, *i.e.*,

TABLE II. Comparison of 2 Types of Tests, Cell Culture (CC), and Embryonated Egg (EE).

Category	No. of tests Monkey H	Monkey M
CC positive EE "	1	15
CC positive EE negative	0	1
CC negative EE positive	2	3
CC negative EE "	4	5

Doubtful tests are scored as negative. Tests with frozen specimens (negative) are excluded.

washing and swabbing (Table III). It should be recalled that in all cases the eye was first washed and then swabbed. In 5 instances the specimen obtained by washing was positive when the swab specimen was negative or doubtful, although positive swab specimens usually produced more inclusions than did the specimens taken by washing. No swab specimens were positive when the washing was negative, and the 2 methods agreed in the remaining 20 instances.

Of the 4 cell cultures of each specimen, tubes 1 and 2 were often fixed and stained first, and when they contained inclusions the cells of tubes 3 and 4 were not examined but were used for passage. Passages were attempted in similar McCoy cultures and in eggs with the resuspended and sonicated cells and/or supernates of tubes 3 and 4 (often combined with the supernates of tubes 1 and 2). In no instance were inclusions found in a second passage to McCoy cells, a result

TABLE III. Comparison of Results with 2 Types of Specimens, Washing (W), and Swab (S), in 2 Monkeys.

Category	No. of tests In cell culture	In yolk sac
W positive S "	6	7
W positive S negative	3	2
W negative S positive	0	0
W negative S "	4	3

Doubtful tests are scored as negative. Tests with frozen specimens (negative) are excluded.

compatible with the general observation that trachoma strains, with few exceptions, are not readily passed in cell cultures. However, passage to eggs of positive cell culture harvests (cells and supernates) resulted in recovery of virus in 5 of 10 attempts. Supernatant fluids alone were positive once when passed to eggs, and negative in 5 cases.

Bacteria were recovered on both kinds of plates, aerobic and CO_2, usually the same type on both plates; occasionally an isolate appeared on one plate only. Table IV records

TABLE IV. Results of Bacterial Culture of Specimens.

Type of bacterium	No. of specimens positive
Staphylococcus, hemolytic	24
" , non-hemolytic	20
Corynebacterium	12
Neisseria	6
Coliforms	3
Bacillus	3
Streptococcus	2
Sarcina	2

types of bacteria and number of specimens in which each was found. The total number of colonies on a plate was often too great to count (exceeding 300), giving estimates of more than 3000 bacteria per ml for 17 of the 30 specimens satisfactorily cultured. No difficulty was encountered with bacterial contamination in either cell culture or yolk sac with the levels of ristocetin and streptomycin used. Fungal contamination of yolk sacs was seen on several occasions and, as noted in a footnote of Table I, prevented completely satisfactory egg tests with specimens of monkey M of days 5 and 42.

Discussion. The observation has been made repeatedly that continued passage of trachoma strains in cell lines has usually not been successful. Nevertheless, a first cycle of growth in cell cultures is generally possible. The results here described indicate that the method used, centrifugation of infective material onto cell monolayers with observation of resulting intracellular inclusions in the first developmental cycle, may be useful in the study of experimental simian trachoma. An earlier study(12) indicates a similar use in human infection with labora-

tory-established virus. The report cited describes the demonstration of trachoma inclusions in cell cultures inoculated with specimens taken on the day following onset of an acute conjunctivitis in a laboratory technician who had accidentally contaminated his face with infected yolk sac emulsion. The culture was shown to be positive 48 hours after inoculation (72 hours after onset) and before ocular signs indicative of trachoma had appeared. We have not yet been able to evaluate the method for diagnosis of naturally-occurring trachoma, although available evidence suggests that it may prove to be useful.

The potential advantages of the cell culture method over yolk sac culture are speed, less chance of interfering bacterial contamination, and greater constancy of the host cell system. In the present tests specimens were first demonstrated to be positive in yolk sac culture from the 10th day after inoculation of the first passage (minimum time) to the 11th day of the second passage (maximum time). In contrast, 48 hours at 35°C appears to be a satisfactory period of incubation of inoculated cell cultures. In other experiments in which large numbers of staphylococci and *Escherichia coli* were added to infected cell cultures to test the capacity of the stated levels of antibiotics to control the contamination, inclusions were demonstrable even when bacterial growth or persistence in the culture was not entirely controlled. It appears that the bacterial load of the inoculated material would have to be very high, or particularly resistant to the antibiotics employed, before the test would be seriously interfered with. When one considers the possible variability in eggs from different sources, and especially the seasonal period described by Jawetz(13) and others in which eggs may be found completely unsatisfactory for growth of trachoma virus, the advantage of using a standard cell line is obvious.

By present methods the cell culture appears to be somewhat less sensitive in detecting virus than is the yolk sac, and this discrepancy might be increased if further blind passages in eggs had been performed. On the other hand, it is possible that alterations in methods can be found that will increase the sensitivity of the cell culture method.

Summary. Ocular specimens, taken repeatedly from experimental trachoma in 2 rhesus monkeys, were cultured in parallel in McCoy cell cultures and in yolk sacs of embryonated eggs, to explore the feasibility of using the cell culture method of demonstrating virus as an aid in recognizing the infection. The results indicate a potential usefulness of the cell culture method in which the test can be read in 48 hours. When washing of the conjunctival sacs was compared with swabbing with dry cotton as a method of obtaining material for culture, the former procedure yielded a slightly greater number of positive tests. Bacteriologic studies on the specimens showed that the levels of streptomycin and ristocetin used in the cell cultures and yolk sacs satisfactorily protected against contamination by a variety of bacterial types.

The technical assistance of the following is gratefully acknowledged: F. H. Spofford, HMC, P. H. Hill, HMC, A. M. Jones, HM3, B. L. Ward, R. Grays.

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Received October 31, 1962. P.S.E.B.M., 1963, v112.

Successful Cultivation of Spleen Fragments in Organ Culture.* (28005)

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Since the description of an organ culture system by Strangeways and Fell(1) a number of investigators have described methods for short term cultivation of hematopoietic and other tissues. With many of these procedures the specimen is placed at the gas-liquid interphase in suitable culture medium so that it has maximum contact with the gas phase yet has liquid exchange with the medium.

Parker(2) maintained rabbit spleen fragments for 4 days in a fluid medium with 80% oxygen in the gas phase. Trowell(3) kept rat lymph nodes for 4 days on cotton wool soaked with serum-saline medium in a gas phase of pure oxygen and also cultivated tissues on lens paper suspended by a tantalum wire grid over a synthetic medium(4). His attempts to cultivate thymus, spleen and bone marrow were unsuccessful(4). Chen(5) described the differentiation of rat embryo spleen in 8 days on lens paper floating on medium composed of fowl serum and chick embryo extract.

This report concerns successful culture of spleen fragments for prolonged periods by a modification of the organ culture technic.

Materials and methods. Fourteen- to 28-day-old Swiss mice were anesthetized by intraperitoneal administration of sodium pentobarbital and their abdomens opened aseptically. After exsanguination from the abdominal aorta their spleens were removed, placed in a sterile Petri dish containing several drops of Dulbecco's phosphate buffered saline(6), and cut into approximately 2 mm diameter fragments.

Sterile tetrafluorethylene (Teflon®) rings, 1.6 cm in diameter and 0.6 in thickness with four 2 mm side holes, were placed in sterile 10 ml beakers and 1 ml of medium was added. The medium consisted of 80% 199 solution and 20% calf serum (Difco) with antibiotics at concentrations of 100 units penicillin and 50 μg streptomycin per milliliter. The pH of the medium was adjusted to 7.2 with $NaHCO_3$. Each beaker was tilted and a 1.8 cm diameter circular piece of ultra-violet sterilized lens paper (Fisher No. 11-996) was then quickly soaked in the medium, placed over the top of the ring and the beaker leveled. The tilting during placing of the lens paper was required to prevent air bubble formation. A spleen fragment was placed on the lens paper, cut surface down, and each beaker was sealed with a No. 5 "cafe au lait" rubber stopper and incubated at 37°C. When the

FIG. 1. Organ culture of spleen fragment.

FIG. 2. Spleen fragment, after 6 days cultivation. Note reticulum cell hyperplasia, medium and small lymphocytes with vesicular and pyknotic nuclei. H & E stain. 375 ×.

FIG. 3. Spleen fragment, after 30 days cultivation. Note lymphoid follicles. H & E stain. 60 ×.

FIG. 4. Spleen fragment, after 30 days cultivation. Central area of lymphoid follicle, demonstrating paired vessels, lymphocytes at various stages of maturity, and scattered plasma cells. H & E stain. 180 ×.

FIG. 5. Spleen fragment, after 30 days cultivation. Margin of lymphoid follicle, demonstrating megakaryocyte, reticulum cells, lymphocytes, plasma cells. H & E stain. 900 ×.

FIG. 6. Imprint of spleen fragment, after 16 days cultivation, demonstrating plasma cells. Wright-Giemsa stain. 900 ×.

* Supported by American Lebanese Syrian Associated Charities (ALSAC) and by a grant from Nat. Cancer Inst., U.S.P.H.S.