The final patient was very upset, wept, and tried to get out of bed. She found it difficult or seemed unwilling to explain what was the matter. She said that her upper limbs seemed numb and as if they did not quite belong to her. There was no similar feeling in the lower limbs. She tried to bite her left arm several times as if to convince herself that it really belonged to her. Approximately 1¼ hours after the medication had been given, 50 mg of meperidine were given intravenously and within less than 10 minutes her condition was greatly improved although she still felt "peculiar." By 3 hours after medication the patient was walking around the ward in good spirits. In none of these patients was there any significant fluctuation in blood pressure, respiration, or pulse.

Discussion. At the dose employed in this study, dexoxadrol is an analgesic with a high incidence of untoward side effects, including some that might be called psychotomimetic. One question that immediately arises is whether or not the "analgesia" is really produced by the distracting side effects. This seems unlikely, in view of these facts: 1) very few patients complained of dramatic side effects, 2) complete or partial relief was reported in patients who had no side effects of any kind, and 3) some patients had side effects without obtaining any relief. On the other hand, the highest incidence of side effects of any kind did occur in the 33 patients who obtained complete relief at one or more interview points after administration of dexoxadrol. Fifty-eight per cent of these patients had one or more side effects from the drug, as contrasted with 29 and 31% respectively for the patients who achieved only partial relief during the 4 hours after dexoxadrol, or obtained no relief during this period.

These results seem of interest for at least two reasons. First, the clear-cut analgesia apparent in our study would not have been predicted from animal experimentation, since the laboratory tests suggested, if anything, a *decreased* tolerance to pain. Second, the psychotomimetic effects seen after dexoxadrol are reminiscent of the analgesic and psychotomimetic properties coexistent in such drugs as LSD(2) and as nalorphine(3), cyclazocine (4), and other narcotic antagonists. In this context, anti-morphine effects of dexoxadrol have been reported in mice(1).

Summary. Dexoxadrol, a compound which in animals appears to increase sensitivity to noxious stimuli, and to antagonize certain effects of narcotic analgesics, has been shown capable of relieving clinical pain. Unfortunately, at the doses employed in this study, the analgesic properties are associated with a high incidence of side effects, including psychotomimetic states. The coexistence of analgesic and psychotomimetic properties in dexoxadrol adds this drug to a list of other agents sharing these properties.

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Received October 1, 1964. P.S.E.B.M., 1965, v118.

Isolation of the Trachoma Agent in Cell Culture.* (29841)

F. B. GORDON AND A. L. QUAN

Department of Microbiology, Naval Medical Research Institute, Bethesda

After establishment of isolates of the trachoma agent in the yolk sac of embryonated eggs, infection of cell cultures can be readily

* Bureau of Medicine and Surgery, Navy Department, Research Task MR005.09-1200.05. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

accomplished, as indicated by appearance of inclusions(1). Growth of the agent in cell culture, however, is limited, and serial passages are regularly successful only when sonication(2) or some other method(3) is used to break up the infected cells to release infectious particles. In experiments with yolk-sac established strains of agents of the trachoma (TRIC) group, we found (unpublished observations) that the large cells produced by gamma irradiation of McCoy cell (McC)(4) cultures are considerably more susceptible to TRIC strains than are unirradiated cells. The greater susceptibility is indicated by more inclusions per microscopic field, larger inclusions, detection of infectivity in higher dilutions of inoculum, and a greater infective titer of harvests from irradiated cultures.

Irradiated McC cells, in addition to embryonated eggs, were therefore used in tests for trachoma agent in a series of conjunctival specimens from suspected trachoma cases, obtained in Taiwan in collaborative investigations with personnel at Naval Medical Research Unit No. 2, Taipei. That study, concerned primarily with a comparison of cell culture and yolk sac as means of detecting trachoma infection, will be reported elsewhere. In several instances in the above study, the infective agent, demonstrated in initial McC culture by microscopic examination, was passed serially in these cells, and isolates were thus established without recourse to the embryonated egg. These results are reported herewith.

Materials and methods. The McC cell line, initiated with human synovial cells, was obtained from Morris Pollard and maintained on Eagle's medium with 10% horse serum. When used for infected cultures, an additional 30 μM of glucose per ml was added, because of the greater susceptibility of cultures maintained in such a medium (GM) as reported previously in preliminary form (5). The culture fluid also contained 200 μg ristocetin and 100 μg streptomycin per ml. The cultures used in these studies consisted of cells that had received 5000 r of gamma radiation from cobalt-60 six to 10 days (usually 7) earlier, and had been transferred in sufficient numbers to flat bottomed tubes containing 12 mm circular coverslips to allow a complete monolayer to form. Such monolayers consist of non-multiplying cells some 2 times as large in linear dimensions as are unirradiated cells, with many possessing multiple, or multilobed nuclei. Inoculation of cultures, with 0.5 ml of specimen, was accompanied by horizontal centrifugation for 1 hour at 1600 $\times$ g, as described previously (6), to increase the degree of infection.

Four cultures were used for each specimen initially and for each passage. After 48 hours incubation at 35°C one of the coverslips was fixed in methanol, stained with a water-alcohol solution of 5% I_2 and KI, and examined as a wet mount. When this culture was found positive, as determined by the presence of inclusions, the remaining tubes of the set were allowed to incubate another 1-3 days. At 3, 4 or 5 days after inoculation, 0.5 ml of fresh GM was added to each tube and the cells were suspended by vigorous agitation on a Vortex Jr. Mixer.† The cell suspensions were pooled and treated for 5 minutes in a sonic oscillator.‡ After dilution, passage was made to irradiated McC cultures in the manner described above, 0.5 ml per tube, and the series was continued. The total dilution between passages was regularly 1:10. Uninfected control cultures were set up at each passage.

A 0.5 ml portion of each diluted cell culture harvest was also inoculated into the yolk sac of each of four 6-day embryonated eggs. These were observed for embryo deaths, and smears of the yolk sacs of dead embryos (or of embryos living at 14 days after inoculation) were stained by a modification of the method of Machiavello and examined. Smears were scored for prevalence of elementary bodies on a 0, 1+, 2+, 3+, or 4+ scheme, and the averaged scores were expressed numerically.

Passage from cells was usually made immediately after harvest, but when storage was necessary, equal volumes of phosphate buffer containing 0.4 M sucrose were added to the infected cell harvests and the mixture was

† Scientific Industries, Inc.

‡ Raytheon, Model DF101.

stored at −60°C. To avoid damage to cells by 0.2 M sucrose when the stored material was passed, it was first diluted 10-fold with GM, then 2.5 ml was used as an inoculum, and after centrifugation of the inoculated tubes, the fluid was replaced with the usual 0.5 ml of GM.

Estimations were made of the number of infective particles in the inocula by counting inclusions at a magnification of 200 ×. The inclusions are differentially stained with iodine by virtue of their glycogen content. When inclusions were scarce the entire coverslip was scanned, but when infection was heavy a smaller portion of the monolayer was examined, usually 30 fields. The counts as given in the Tables are estimates of the numbers of inclusions in entire monolayers (12 mm diameter), many obtained by use of the appropriate factor. In previous studies (to be published) we have confirmed the validity of iodine-stained inclusion counts as a means of quantitation of infectivity, as described by Furness *et al*(2).

Results. The cultures of 5 specimens, shown to be positive in the first passage, were used for initiating 7 series of passages, as described, which were successful in all instances.

In 5 series passage was regularly made at intervals of 3 or 4 days, often alternating. These isolates, following recommended procedures(7), are now designated as TRIC/ /TW/NMR-6/OT, TRIC/ /TW/NMR-13/ OT, TRIC/ /TW/NMR-15/OT, TRIC/ /TW/NMR-17/OT, TRIC/ /TW/NMR-19/OT, of which the shortened forms appear elsewhere in the text and in the Tables. In 2 instances first passage harvests were also used to initiate additional parallel series with passage at 5-day intervals. The methods for these 2 series were as described except for one added maneuver. The cells at each passage were suspended at 72 hours, by agitation with the Vortex Mixer and immediately reset, with centrifugation, in the same tubes. This procedure (without centrifugation) has been found useful in maintaining chronically infected flask cultures(8). In these experiments it appeared to have no advantage.

Detailed data on one of the series (NMR-19, passed at 3- and 4-day intervals) are given in Table I. It may be seen that inclusion counts increased markedly as the series progressed, beginning at 41, reaching 1200 by the 6th passage, approximately 17,000 by the 9th and 10th, and nearly 50,000 at the 11th (passage 11a). A summary of inclusion counts only is given for the other series in Table II. For more accurate quantitation we use the counts of 3 or 4 coverslips; these single counts, however, with many rounded off to the nearest 10 or 100, clearly indicate the trend. In a few instances there have been marked reductions in counts between passages, the reason for which is unknown. These are seen at passage 12 in Table I, and at passage 9, for example, in the 3rd column of Table II. These can only be ascribed to some unrecognized adverse factor.

The relatively low and irregular counts seen in the two series passed at 5-day intervals (Table II) indicate that one or both of the methods used in these, different from the other series, gives relatively poor results. At some passages it was noted that the cells were in unsatisfactory condition at the 5th day.

We have observed in previous work that TRIC inclusions in very heavily infected monolayers may not take the iodine stain, and this occurred in certain passages noted in the Tables. The infection doubtless was at a high level in these passages as judged by the inclusion count of the previous passage, or by a cytopathic effect (CPE). The high incidence of inclusions was confirmed by microscopic observation. The inclusions could be recognized, when in profusion in the iodine stained monolayer, by their morphologic characteristics even though they were not differentially stained. It may be postulated that glycogen may not be formed in heavily infected cultures because of an insufficient amount of some essential ingredient.

Deterioration of cell monolayers was seen occasionally and it was obvious, from comparison with uninfected control cultures that a characteristic abnormality could be attributed to the presence of the infection. The lesion is illustrated, in comparison with an

uninfected culture, in Figs. 1 & 2. The cells tended to clump, producing drawn-out cellular processes with spaces between, giving the entire monolayer a lacy appearance. This was sometimes accompanied by release of cells from the glass surface. The degree of this cytopathic effect did not appear to be completely correlated with numbers of inclusions, although it appeared only when the count was high, including most of those cases where the inclusions appeared not to contain glycogen.

After these series were started we noted a report(9) from Russia that describes the isolation of an agent from trachomatous conjunctival scrapings by serial passage in chick fibroblast cells *in vitro*. Inclusion bodies were seen in the third passage and the series was continued through 21 passages in these cells. Seventh passage material was passed intra-

TABLE I. Data on Serial Passage of Cell Culture Isolate NMR-19 in Irradiated McCoy Cells, and Lateral Passage to Yolk Sacs.

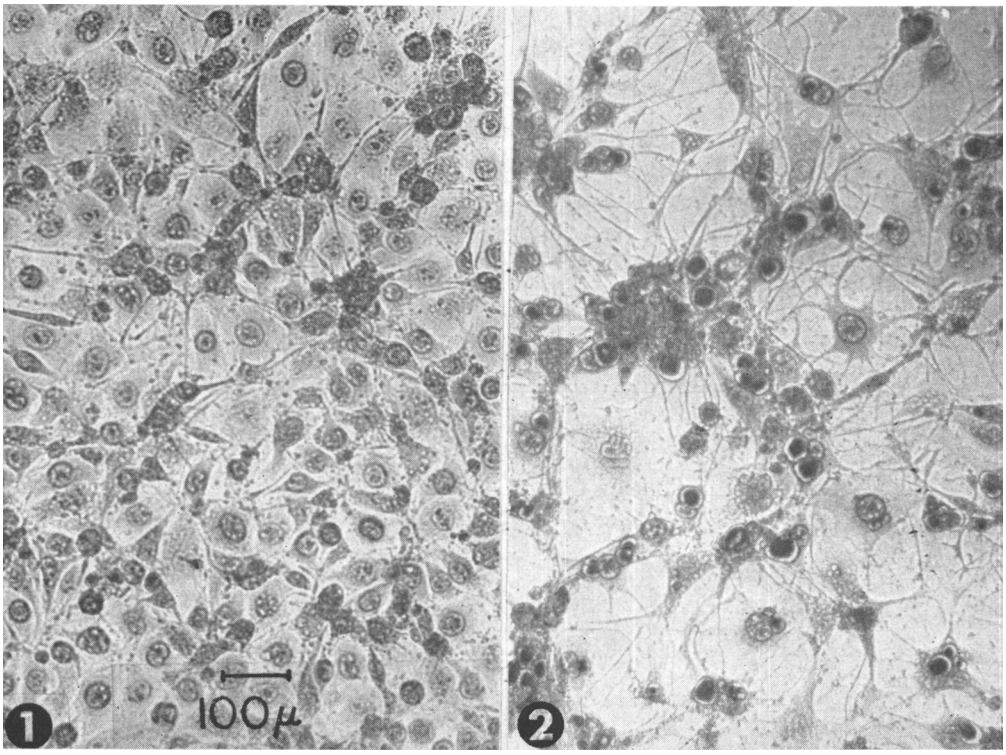
Passage No.	Dilution	Result in cell culture (inclusion count)	Result in y. sacs D/T	Result in y. sacs E.B. score	Interval to harvest of cell culture (day)
1		41	0/4	1.0	3
2	1:10	42	0/4	2.0	3
3	"	102	1/4	4.0	4
4	"	113	0/4	1.0	3
5	"	209	3/4	3.8	4
6	"	1,200	3/4	2.8	3
7	"	1,180	2/3	2.5	4
8	"	4,900	0/4	.5	3
9	"	16,900 (CPE)	2/4	1.5	4
10	"	17,000	3/4	.75	3
11	"	I.N.	2/3	2.3	4
11a*	1:8	46,700	1/4	.5	3
12a	1:20	I.N. (CPE?)	N.D.	N.D.	–
12	"	241	1/4	1.3	3
13	"	6,300	1/2	.5	4
14	1:10	14,400 (CPE)	2/4	1.8	5
15	1:20	I.N. (CPE)	N.D.	N.D.	–

* = Inoculum had been frozen and stored. Passages 11a and 12a represent a short side branch of the series, initiated with stored harvest of passage 10.
D/T = Embryo deaths/total embryos.
E.B. = Estimation of average relative incidence of elementary bodies in stained yolk sac smears. 4.0 = maximum. See text.
I.N. = Most inclusions were iodine negative and could not be counted satisfactory (see text).
CPE = Evidence of cytopathic effect. See text.
N.D. = Not done.

TABLE II. Counts of Inclusions per Coverslip in Serial Passages of Trachoma Agent Isolates in Cell Cultures.

Passage No.	Passed at 3 and 4 day intervals NMR-6	NMR-13	NMR-15	NMR-17	Passed at 5 day intervals NMR-13	NMR-19
1	77	8	680	1,100		
2	66*	68	650	247	†	697
3	3,120	35	930	786	167	1,330
4	12,400	120	2,550	2,690	191	133
5	6,210	590	3,880	4,200	242	1,180
6	3,880	480	2,300	4,640	145	763
7	19,900	2,300	1,300	6,980	25	1,240*
8	15,200	1,100	2,700	7,100	73	1,220*
9	16,400	130	5,800	17,000	28*	807*
10	—	610	11,800‡	26,900‡	16*	I.N.*‡

* Inoculum had been frozen and stored.
† Inclusions present, but not counted.
‡ Evidence of CPE.
I.N. Most inclusions were iodine negative and could not be counted satisfactorily (see text).



FIGS. 1 & 2. Irradiated McCoy cell monolayers, stained with iodine solution. Magnification is the same in each. 1. Uninoculated. 2. Inoculated 48 hours earlier with NMR-19, 8th cell passage. Note CPE and numerous heavily stained cytoplasmic inclusions.

cranially into mice and brain passages in these animals were continued. At the seventh passage mice died and elementary bodies were seen in brain smears. We made one attempt to adapt our strain NMR-19 to this host by passing a harvest of the 10th cell culture passage to mice by the intracerebral and intranasal routes. Two further (blind) passages were made with brain and lungs, with negative results.

The results of testing each cell culture harvest by yolk sac inoculation as recorded in Table I, showed an irregular pattern, with little apparent correlation between the severity of infection in the embryos and the inclusion count in monolayers, produced by the same inoculum. Similar data for the other passage series took the same pattern. It is clear that adaptation to the yolk sac did not occur during the series of passages in McC cultures.

Comment. These results indicate that it is entirely feasible to isolate and establish TRIC agents in cell cultures only, without use of the chick embryo. A later publication will evaluate this cell culture method, in comparison with inoculation of yolk sacs, for demonstration of the infection in conjunctival specimens.

Inoculation of cell culture harvests, throughout 10 or more passages, into yolk sacs gave the same sort of irregular result usually seen in the 1st or 2nd yolk sac passage from conjunctival specimens. Once an isolate has been established in yolk sacs regular deaths of embryos with 3+ or 4+ yolk sac smears is the rule. A preparation of an established yolk sac strain, with an infectivity great enough to produce 10,000 or more inclusions per coverslip, would be expected to kill embryos promptly and regularly in contrast to the results in these series. It can be assumed, therefore, that these cell culture isolates and yolk sac isolates are qualitatively different with respect to the two host systems, as a result of adaptations.

Whether they differ in any other significant way, and whether the cell culture isolates possess useful characters not seen in previous isolates, is not presently known.

Summary. Five isolates of the trachoma agent have been made by culture in irradiated McCoy cells, without use of the standard method of yolk sac inoculation. These have been passed serially in cell culture at intervals of 3-4, or at 5 days, 9 to 15 times. Increase in level of infection was indicated by increasing counts of inclusions observed regularly at 48 hours after inoculation, and by cytopathic effect in some passages. Passage of cell culture harvests to yolk sacs gave irregular results throughout the series with no indication that successful passage in cell culture was accompanied by adaptation to yolk sac.

The technical assistance of C. Thomas Colonna, J. I. Thomas, and Richard Grays is gratefully acknowledged.

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Received October 7, 1964. P.S.E.B.M., 1965, v118.

Immunological Properties of Bacterial Cell Wall Mucopeptides.* (29842)

ESSA M. ABDULLA AND JOHN H. SCHWAB (Introduced by John K. Spitznagel)
Department of Bacteriology and Immunology, University of North Carolina, Chapel Hill

The mucopeptide component of bacterial cell walls is a common entity among a wide variety of microorganisms and is essential for the rigid structure of the cell wall. There have been several analyses showing qualitative and quantitative variations among microorganisms in the composition of this component(1). To our knowledge there have been no immunochemical studies on mucopeptides, although others have worked with antigens containing this material(2,3). This report presents evidence for the antigenicity of mucopeptides from beta-hemolytic streptococci and describes the mucopeptide-serum reaction. The antigenic relationship between mucopeptides from representative bacterial species, and the structure of the antigenic sites, will be reported later.

* Supported by USPHS Research Grant AI-00949 from Nat. Inst. of Allergy & Infect. Dis.

Materials and methods. Cell walls were prepared by the method of Salton and Horne (1). Some preparations were further purified by sucrose gradient centrifugation(4). Treatment included digestion with trypsin and ribonuclease. Mucopeptide was obtained by formamide extraction as described by Krause and McCarty(5). The extraction was repeated 3 times at 170°C for 60 minutes. Preparations were also made from some strains by trichloracetic acid (TCA) extraction(6) or the warm phenol method(7). The following bacteria have been used: group A streptococcus, type 3, strains D-58 and C203/29/4; group A variant streptococcus, strain B455; group C streptococcus, strain H46A; group D streptococcus, strain F-24; *Staphylococcus albus; Bacillus cereus* and *Chromobacterium violaceum.*

Rabbits were immunized by one of 3 meth-