than one lipid component. More detailed studies along this line are now in progress.

From the foregoing it is apparent that it has been possible to obtain from the cell bodies of the group A streptococcus a non-polar lipid fraction highly active in suppressing the development of ascitic tumor when pre-incubated with tumor cells before inoculation. Although it cannot be said with certainty that this non-polar lipid fraction represents the sole component responsible for the anti-tumor activity of the group A *Streptococcus* demonstrated by KOSHIMURA et al.[1], the present results are of special interest in view of the earlier investigations that the lipid preparations derived from royal jelly[15] and *Sh. flexineri* and *E. coli*[16] have been capable of inhibiting the development of experimental tumors in animals. The possible importance of the streptococcal lipids as anti-tumor agents remains to be evaluated.

Résumé. On a isolé chez la souris, d'une souche de *Streptococcus hemolyticus* une fraction lipidique qui inhibe complètement le développement de la tumeur ascitique d'Ehrlich quand la fraction lipidique est préincubée avec les cellules de la tumeur avant inoculation.

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Transfer of R-Factor Mediated Aminoglycoside Antibiotic Resistance in the Allantoic Cavity of Chick Embryos

The discovery of R-factor (episome)-mediated, transferrable, multiple-drug resistance among various species of *Enterobacteriaceae* and *Pseudomonadaceae*[1] prompted several investigators to examine a variety of laboratory animals with regard to their suitability for in vivo transfer of multiple-drug resistance[2–8]. Generally, germ-free animals, infantile animals, or antibiotic-treated-modified conventional adult animals had to be utilized for these experiments to succeed. Reported also was transfer of multiple-antibiotic resistance from *Escherichia coli* of animal or human origin to resident *E. coli* within the gastrointestinal tract of a human volunteer, following oral administration of drug-resistant donor organisms[9]. Here we wish to report in vivo transfer of R-factor-mediated aminoglycoside antibiotic resistance from 2 clinical enterobacterial isolates to a drug-sensitive, recipient strain of *E. coli* within the chick allantoic cavity.

Multiple drug-resistant isolates *E. coli* 1531 and *Klebsiella pneumoniae* 829, from clinical sputum specimens[10], served as donors; the recipient in all experiments was *E. coli* K-12, strain 1485 (F^- lac^+), resistant only to nalidixic acid (*E. coli* 1485-Na-R). Stock solutions of kanamycin sulfate (2,000 µg/ml Km; Bristol Laboratories, Syracuse, N.Y.) and nalidixic acid (5,000 µg/ml Na; Sterling-Winthrop Research Institute, Rensselaer, N.Y.) were prepared in sterile distilled water and 0.1 *N* NaOH, respectively, and sterilized through membrane filtration (0.22 µm; Millipore Filter Corp., Bedford, Mass.). Disc antibiograms of donor, recipient, and transcipient organisms were determined with a standardized technique[11]; broth dilution tests were performed as described previously[12]. The donor organisms tolerated greater than 100 µg/ml Km and were sensitive to Na; *E. coli* 1485-Na-R was inhibited by 3 µg/ml Km and tolerated greater than 100 µg/ml Na.

Transfer of aminoglycoside antibiotic resistance from the donor organisms to *E. coli* 1485-Na-R in vitro was accomplished by the technique of ANDERSON and LEWIS[13,14]. Samples (0.05 ml) from co-cultivated organisms (1.5×10^7 donor and 1.5×10^8 recipient organisms/ml in a total of 20 ml nutrient broth at 0 time; incubated at 35 °C for 18 h) and control donor and recipient cultures were spread on MacConkey agar (Difco) plates containing 20 µg/ml Km (MAC-Km), 50 µg/ml Na (MAC-Na), 20 µg/ml Km + 50 µg/ml Na (MAC-Km-Na), or no drug (MAC). Plates were incubated at 35 °C for 24 h and examined for the presence of transcipients[15], which were subcultured to MacConkey agar, identified biochemically, and disc diffusion susceptibility tested.

For transfer of drug resistance in vivo, groups of 3 viable, 8-day-old, specific-pathogen-free chick embryos (Truslow Farms, Inc., Chestertown, Md.) each were inoculated into the allantoic cavity with 0.2 ml of the donor-recipient mixtures (the organisms were mixed in the same ratio as above immediately prior to inoculation), as well as organisms in isotonic saline, respectively. Control embryos received saline alone. Following incubation at 37 °C for 18 h, the chick embryos were candled, survivors were chilled for 1 h, and 0.05 ml aliquots of harvested allantoic fluid were spread on plain and selective MacConkey agar plates. Transcipients were processed as above.

In vitro transfer of aminoglycoside antibiotic resistance from *E. coli* 1513 (I) and *K. pneumoniae* isolate 829 (II) to *E. coli* 1485-Na-R (III) was readily achieved (Table I); resistance markers to Km and neomycin were transferred regularly, while resistance to streptomycin was transferred irregularly. Similar results were obtained when bacterial conjugation took place within the allantoic

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Table I. Disk antibiograms of donor and recipient control organisms, in vitro and in vivo-derived transcipients[a]

Organisms	Ampicillin	Cephalothin	Chloramphenicol	Gentamicin	Kanamycin	Nalidixic acid	Neomycin	Nitrofurantoin	Polymyxin B	Streptomycin	Tetracycline	Triple sulfonamide
Controls:												
I = *E. coli* 1531 (donor)	R	S	S	S	R	S	R	S	S	R	R	R
II = *K. pneumoniae*, 829 (donor)	R	S	R	S	R	S	R	S	S	R	R	R
III = *E. coli* 1485-Na-R (recipient	S	S	S	S	S	R	S	S	S	S	S	S
In vitro:												
I × III transcipients	S	S	S	S	R	R	R	S	S	R	S	S
II × III transcipients	S	S	S	S	R	R	R	S	S	S	S	S
In vivo:												
I × III transcipients	S	S	S	S	R	R	R	S	S	R	S	S
II × III transcipients	S	S	S/R[b]	S	R	R	R	S	S	S/R	S/R	S/R

[a] S and R denote sensitive and resistant, respectively[11]. [b] S/R designates variable transfer of resistance to particular drug.

Table II. Quantitative results (colony counts) of in vitro and in vivo conjugation of *E. coli* 1531 and *K. pneumoniae* 829 with *E. coli* 1485-Na-R

Organisms crossed	Paired organisms and transcipients				Donor control				Recipient control			
	MAC-Km-Na	MAC-Km	MAC-Na	MAC	MAC-Km-Na	MAC-Km	MAC-Na	MAC	MAC-Km-Na	MAC-Km	MAC-Na	MAC
In vitro[a]:												
I × III	27[b]	TNTC[c]	TNTC	TNTC	0	TNTC	0	TNTC	0	0	TNTC	TNTC
II × III	90	TNTC	TNTC	TNTC	0	TNTC	0	TNTC	0	0	TNTC	TNTC
In vivo[a]:												
I × III	> 200	TNTC	TNTC	TNTC	0	TNTC	0	TNTC	0	0	TNTC	TNTC
II × III	> 200	TNTC	TNTC	TNTC	0	TNTC	0	TNTC	0	0	TNTC	TNTC

[a] I = *E. coli* 1531 (donor); II = *K. pneumoniae* 829 (donor); and III = *E. coli* 1485-Na-R (recipient). [b] Number of transcipient colonies/plate. [c] TNTC, too numerous to count.

cavity of chick embryos (lower third of Table I), except that in this system *K. pneumoniae* (II) irregularly transferred resistance to chloramphenicol, tetracycline and triple sulfonamide to *E. coli*-1485-Na-R. Counts of transcipients obtained from typical in vitro and in vivo experiments are listed on Table II.

Following the addition (1.5×10^8 organisms/ml) of *Staphylococcus aureus*, enterococci, *K. pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, a combination of these 5 bacterial species, or the addition of 'normal human fecal flora' (growth obtained from 1 g feces in thioglycollate broth, incubated at 35 °C for 18 h, containing various enterobacterial species, *Clostridium* sp., *Bacteroides* sp., etc.) to the donor-recipient mixtures, no interference was noted.

The finding that the allantoic cavity of chick embryos proved suitable for in vivo transfer of R-factor-mediated drug resistance was expected. For all practical purposes, the chick embryo may be considered as free of bacteria. Thus bacterial conjugation could proceed unimpeded. Of interest was the observation that the – admittedly limited – number of added bacterial species did not interfere with bacterial conjugation within the allantoic cavity. However, premature death (within 18 h following infection) of almost all inoculated chick embryos might not have permitted the added organisms to exert their full interfering metabolic potential within the allantoic cavity[16].

Zusammenfassung. Die Allantoishöhle von 8 Tage alten Hühnerembryonen erwies sich als geeignet für die in vivo-Übertragung der Aminoglykosid-Antibiotika-Resistenz der klinisch isolierten Stämme *E. coli* 1531 und *K. pneumoniae* 829 auf den Rezipienten *E. coli* K-12, Stamm 1485.

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