aggregates. In the mammalian lenses the chains consist of particles of diameter 15–20 nm, while in the bird and frog lenses the particles were of the order of 12–15 nm. A filamentous backbone (6–8 nm in diameter) was observed in all lenses examined. The clustering of particles along the chain usually obscures the backbone, which is best seen in areas free of particles (figure, B). The size of the backbone is consistent with that of actin.

These chain-proteins can be pelleted from the 37,000 × g water-soluble fraction of the lens by centrifugation at 78,000 g for 2 h[2], and represent elements of the intracellular matrix not pelleted by centrifugation of the lens homogenate at 37,000 × g[6]. The chain-proteins are characteristic of the fibre cell, and are not found in the epithelial cell. Previous studies on the chick suggest that crystallin and non-crystallin proteins are present in the chains[6].

It has been suggested that the chain-proteins represent crystallins that have formed a stable association with microfilaments in situ[5]. It appears that with epithelial cell differentiation into fibre cells, lens crystallins became stabilized by association with other proteins into unique morphological structures, forming a cytoskeletal for the cell. The relationship of this structural arrangement to cataract formation remains to be elucidated.

1 This study was supported by research grant EY-01417 of the National Institutes of Health, Bethesda, Maryland, USA.
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Antimicrobial activity of metal derivatives of sulfonamides

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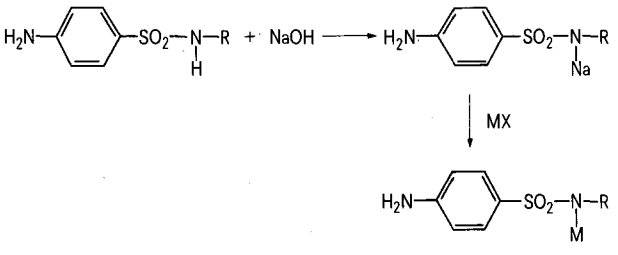
Summary. Antimicrobial activities of a series of metal derivatives of some sulfa drugs were examined. Such metal derivatives showed higher antimicrobial activity than the parent sulfa drugs, and among the metals, gold derivatives are seen to be the most effective.

Sulfonamides have been used for microbial infections, but because of their acquiring resistance and solubility problems, the molecules have been modified for more favorable drugs. Pharmacology of the basic structure of the molecule is well defined, and the molecular site to be modified is limited to the N^1-position. Modification at the aromatic amino group will lead to non-antimicrobial molecules.

In the present study, a series of metal derivatives of some sulfa drugs were made, and their antimicrobial activities were tested. Synthesis and antimicrobial properties of the metal derivatives are described briefly in this note.

Synthesis. General procedures are as follows:



Metal derivatives of sulfonamides and their MIC's for various test bacteria

Test organisms	SD	AuSD	AgSD	ZnSD	MnSD	MgSD	SM	AuSM	AgSM	SI	AuSI	AgSI
Staphylococcus aureus Rosenbach FDA-209-P JC-1	100	50	50	> 100	> 100	> 100	> 100	> 100	25	> 100	> 100	100
Staphylococcus aureus Smith S-424	> 100	50	50	> 100	> 100	> 100	> 100	> 100	100	> 100	> 100	100
Streptomyces faecalis ATCC 8043	> 100	12.5	25	> 100	> 100	> 100	> 100	> 100	50	> 100	> 100	100
Bacillus subtilis ATCC 6633	100	12.5	50	25	> 100	50	> 12.5	25	25	25	25	100
Escherichia coli NIHJ JC-2	> 100	6.25	12.5	50	> 100	50	100	50	50	100	50	50
Escherichia coli K-12 IAM 1264	100	6.25	12.5	100	> 100	100	> 25	50	25	25	50	50
Salmonella typhi O-901-W	100	6.25	12.5	50	> 100	100	> 100	50	25	> 100	50	50
Salmonella enteritidis No. 11 (Toukai)	> 100	6.25	25	> 100	> 100	> 100	6.25	50	50	100	50	100
Shigella dysenteriae Shigae	25	6.25	12.5	25	> 100	25	25	25	6.25	50	12.5	12.5
Klebsiella pneumoniae	25	6.25	25	50	> 100	50	25	50	100	25	50	100
Proteus morganii Kono	25	6.25	12.5	25	> 100	50	> 100	25	25	25	12.5	25
Proteus vulgaris OX-19	100	6.25	12.5	50	> 100	50	> 100	50	25	25	25	50
Proteus species C 73-23	> 100	3.13	12.5	> 100	> 100	> 100	> 100	50	25	> 100	25	50
Serratia species No. 1	> 100	6.25	25	> 100	> 100	> 100	> 100	50	100	> 100	50	100
Serratia species No. 2	> 100	12.5	25	> 100	> 100	> 100	> 100	50	50	> 100	50	100
Pseudomonas aeruginosa IAM-1007	> 100	12.5	12.5	> 100	> 100	> 100	> 100	> 100	50	> 100	> 100	100
Pseudomonas aeruginosa E-2	> 100	12.5	12.5	> 100	> 100	> 100	> 100	100	50	> 100	> 100	100
Vulgaris parahaemolyticus K-5	100	6.25	12.5	100	100	100	50	25	12.5	> 100	> 100	12.5

Metals used are Au, Ag, Zn, Mn and Mg. Legands (sulfonamides) are: SD, sulfadiazine; SM, sulfamethizole; SI, sulfisomidine.

where, R's are substituted groups of sulfonamides, and M's are Au, Ag, Zn, Mn or Mg.

A typical synthesis is illustrated by gold sulfadiazine: that is, 2.5 g (1 mmole) of sulfadiazine, $C_{10}H_{10}N_4O_2S$, is suspended in water at room temperature, and an equivalent sodium hydroxide, solution (0.4 g in 50 ml) is added. A solution thus obtained is stirred while 10% solution of metal salt is added. The solution turned to a brownish suspension, indicating a complex formation; and after completion of adding gold sodium chloride solution, the suspension then formed is stirred for a further 30 min at room temperature, and brownish powder is obtained after centrifugation, drying in vacuum over P_2O_5, with 80% yield. Gold and silver derivatives of sulfa drugs have been characterized by means of UV, NMR, IR and CHN analyses.

Antimicrobial property. Antimicrobial potencies of the compounds were determined as follows:

Preincubation: Medium, Trypticase soy broth (Difco).

Assay: Medium, Mueller hinton medium (Difco).

Microbial suspension of the test organism was incubated in the preincubation medium at 37 °C for 18 h, and then $^1/_{1000}$ diluted suspension obtained by the preincubation was inoculated to the test medium. MIC's of the compounds (μg/ml) for each test bacteria are listed in the table.

Among various metals attached to sulfonamides by coordination, gold and silver showed promizing antimicrobial effects. The effect is enhanced on comparing with the parent sulfonamide compounds.

Some metals are essential to living organisms, some are toxic, and others show mild biological effect. Magnesium, manganese and zinc are utilized by living organisms, so that their coordination to sulfonamides did not enhance antimicrobial property of the parent compounds. Gold and silver themselves show a mild bactericidal property, and when they are coordinated to sulfonamides, the effect is more potent than that of the parent compounds. Toxic metals such as Hg and As should be tested; but, because of their apparent toxicity, we did not use such derivatives for human application.

1 We wish to thank for the material supplied by the Central Research Laboratories, Meiji Seika Kaisha, Ltd, for antimicrobial assay.

Identification of *Trypanosoma theileri* as a contaminant in primary cultures of bovine retina

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Summary. T. theileri has been isolated from primary cultures of bovine retina and subcultered successfully for 2 passages in sub-confluent cultures. When cultures reached confluency no trypomastigotes or epimastigotes could be detected and attempts to recover trypanosomes from these cultures were unsuccessful. The presence of intracellular forms could not formally be excluded.

Although *Trypanosoma theileri* Laveran 1902, is generally nonpathogenic in the bovine, a number of fatal cases have been reported to be caused by this universally distributed organism[2]. In the host the organism appears to localize in a wide variety of organs including the central nervous system (CNS)[3]. Thus, primary cell cultures of calf spleen[4], blood lymphocytes[5], leukaemic lymph nodes[6], and fetal bovine kidney[7] have resulted in an outgrowth of both cells and trypanosomes. To our knowledge the isolation of this trypanosome from cultures of the CNS has not yet been reported. The present report describes such isolation from bovine retinal cell cultures.

Materials and methods. The external surface of bovine eyes, obtained at a local slaughter house, was sterilized by successive dipping in 70% ethanol. An incision was made at a point 1 cm caudal to the dorsal limbus and extended circumferentially parallel to the limbus to obtain 2 portions. The anterior portion was discarded. The vitreous humor was gently removed from the posterior portion to expose the underlying retina. The retina was detached with a rubber policeman from the pigmented epithelium, severed from the papilla and transferred to a centrifuge tube containing 50 ml of Eagle's minimal essential medium (MEM) (final pH 7.2).

Retinal tissue was disrupted into individual cells or groups of 2–10 cells by repeated pipetting (25 times) with a 10 ml pipette, centrifuged (400 × g for 10 min) and pelleted cells were resuspended in 100 ml of MEM + 20% fetal bovine serum (FBS), plated in 1-ml volumes in Linbro plastic tissue culture plates (FB16-24TC) and incubated at 37 °C in a humidified 5% CO_2 atmosphere. The total time elapsed between killing the animal and subsequent plating of the cells was approximately 2 h. The growth and characteristics of these retinal cells will be the subject of a separate communication[8].

When trypanosomes were detected, air dried smears of organisms, in 25% FBS, were made and stained with Giemsa. All measurements were done with an ocular micrometer; organisms were counted using an Improved Neubauer hemocytometer. Subsequent passage of trypanosomes from the primary isolate was carried out in adjacent wells or in freshly prepared cultures. Inocula consisted of 50 μl aliquots of a suspension containing cells and trypanosomes that had been obtained by trypsinization of infected monolayers.

Results and discussion. Routine sterility control on the 2nd day of culture revealed no microbial contaminants. 6 days post plating marked acidity was noticed in 1 of the culture wells; examination of the culture fluid revealed large numbers of motile, flagellated organisms. Inspection of wet mounts under phase microscopy (magnification: × 800) led to a tentative identification of the organism as *T. theileri.* To expedite verification, trypanosomes in stained smears were subjected to micrometer measurements. These results are summarized in the table and the data are comparable to that of others[2,9]. On the basis of these morphological criteria we conclude that the organism is *T. theileri.*

Examination of the forms of the organism in the prelimi-