Chemical and Bacteriological Studies of the Photochemical Reaction Product of Ammonium Hexachloroiridate(IV)[1] (35122)

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Rosenberg *et al.* (1) observed that a solution of the complex ion, hexachloroplatinate ($[PtCl_6]^{2-}$), when irradiated in the presence of NH_4^+ ions undergoes a photochemical reaction in which chloride ions of the ligand are sequentially exchanged for 1, 2, or 3 ammonia molecules. The net charge on the complex decreases by 1 negative charge at each step. These changes can be monitored electrophoretically or spectrophotometrically. Each species of the complex has a different action on *Escherichia coli*. The doubly negative species is bactericidal, the singly negative species is virtually innocuous, and the neutral species inhibits cytokinesis thereby inducing filamentous growth (1). The *cis* isomers of the neutral chloroammino species of platinum(IV) and platinum(II) markedly inhibit development of sarcoma 180 and leukemia L1210 in mice (2); biochemical studies with the neutral species of platinum (II) showed that it exerts a pronounced and persistent inhibition of DNA synthesis in Ehrlich ascites tumor cells *in vivo* (3).

Other group VIIIb transition metal compounds were tested by Rosenberg *et al.* for possible filament-inducing properties, and the most active ones reported were certain compounds of rhodium and ruthenium (4, 5). Ammonium hexachloroiridate was tested and was reported to be bactericidal without selectively inhibiting cytokinesis, while potassium hexanitroiridate was reported to cause no change in bacterial growth rate or cell length.

Iridium is immediately adjacent to platinum in group VIII of the periodic table, and its mass more nearly approximates that of platinum than does that of any other group VIII element. This chemical proximity of iridium to platinum suggests that it too may undergo the type of transformation which Rosenberg *et al.* proposed for platinum (1):

$$[MX_6]^{2-} + nNH_4^+ \xrightarrow{h\nu} [MX_{(6-n)}(NH_3)_n]^{(2-n)-} + nH^+ + nX^-$$

where M = platinum and X = chlorine. Certain chemical and pharmacological properties of solutions of ammonium hexachloroiridate irradiated in an ammonia atmosphere are consequently the topic of this report.

Materials and Methods. Ammonium hexachloroiridate was obtained from Alfa Inorganics, Inc. Solutions for irradiation were prepared in distilled water. Small test tubes containing these solutions (3.0–6.0 mg/ml) were placed into quart Mason jars containing 25 ml of a 3% $(NH_4)OH$ solution, and the jars were closed with standard lids. The solutions were then irradiated approximately 2 in. from a light fixture fitted with 2 General Electric 15-W fluorescent tubes. Absorption spectra were recorded with a Cary Model 15 spectrophotometer and electrophoretic mobilities were detected with a Gelman electrophoresis apparatus operated at 200 V. *Escherichia coli* B (ATCC No. 11303) was grown at 37° in a synthetic liquid medium (6), and optical densities of cultures were measured with a Coleman spectrophotometer at 540 mμ. Photomicrographs were taken with a Leitz Orthoplane apparatus.

Results and Discussion. When solutions of $(NH_4)_2(IrCl_6)$ were irridiated in an ammonia environment, detectable spectral changes occurred in less than 1 hr and progressed to virtually the maximum extent at about 7 hr.

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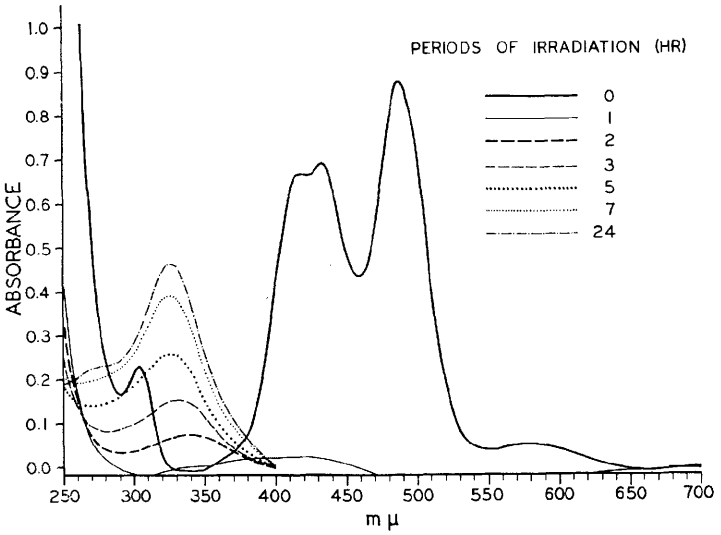


FIG. 1. Absorption spectra of solutions (diluted to 100 μg/ml) of $(NH_4)_2(IrCl_6)$ after irradiation in an ammonia atmosphere for various intervals.

The initial intense brown color diminished until the solutions were almost colorless. Following this relatively rapid change a further alteration occurred and yielded a solution which, undiluted, was violet in color. When these violet solutions were diluted to concentrations appropriate for spectral measurements, no absorption in the visible spectrum was evident, but a new peak occurred at 325 mμ (Fig. 1). When solutions were irradiated

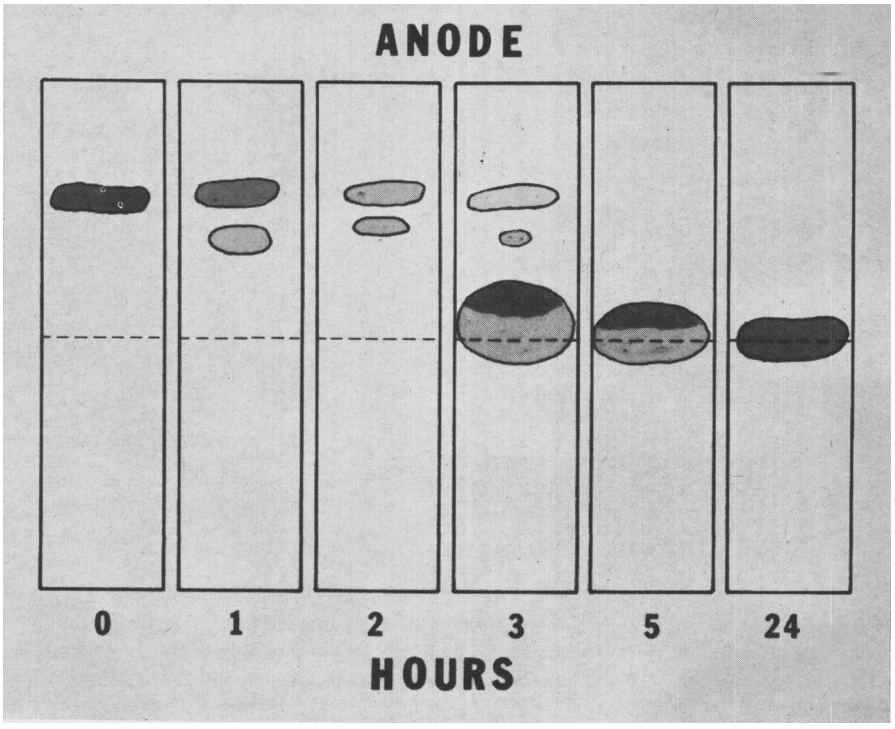
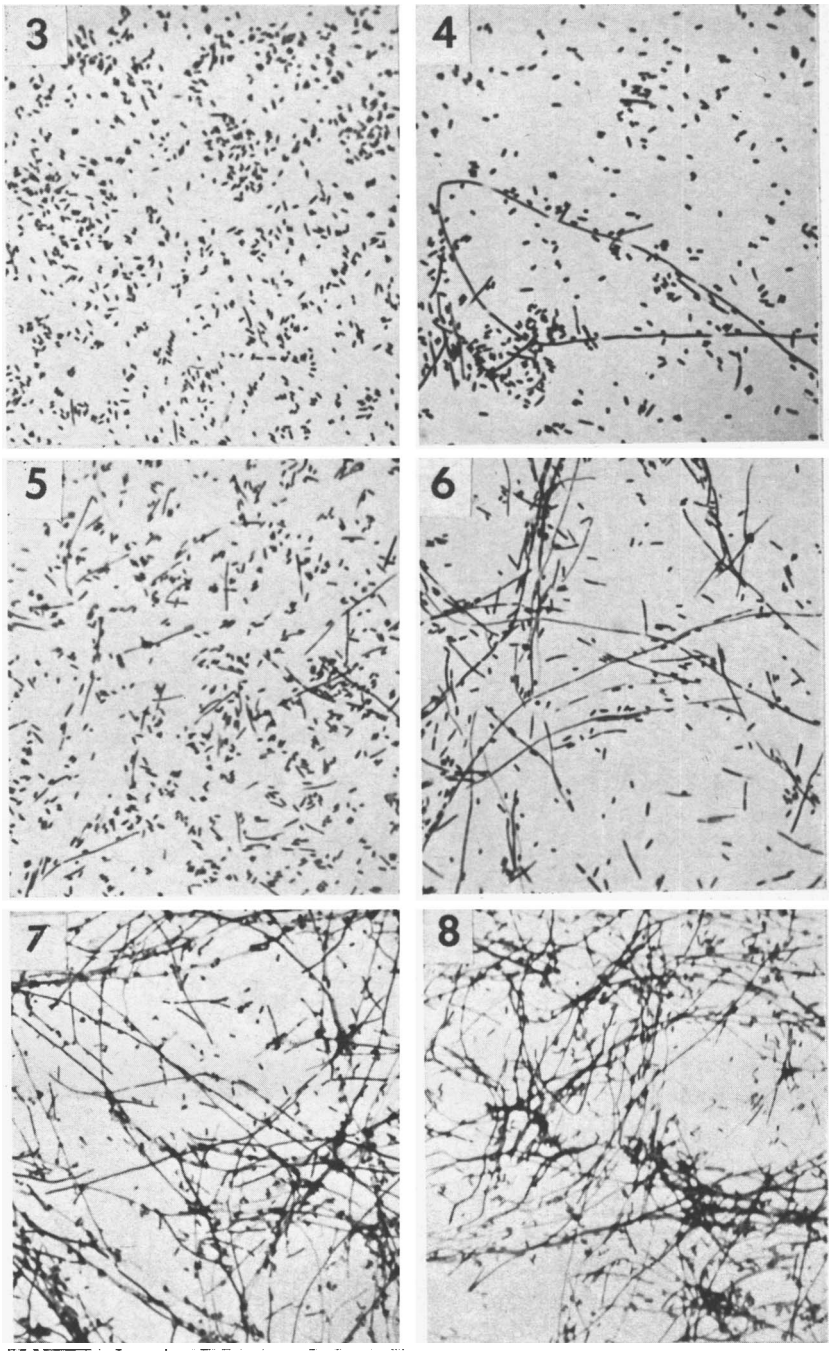


FIG. 2. Diagrammatic depiction of electrophoretic mobility patterns of solutions of $(NH_4)_2(IrCl_6)$ after irradiation in an ammonia atmosphere for various intervals.



FIGS. 3–8. Photomicrographs (× 850) of cells of *E. coli* grown in synthetic medium containing $(NH_4)_2$ ($IrCl_6$) which had been irradiated in solution in an ammonia atmosphere for the following intervals prior to addition to the culture medium: Fig. 3, 0 hr; Fig. 4, 1 hr; Fig. 5, 2 hr; Fig. 6, 3 hr; Fig. 7, 5 hr; and Fig. 8, 7 hr. The final concentrations of the compound [expressed as $(NH_4)_2$ ($IrCl_6$)] in the culture tubes were 10 μg/ml for Fig. 3, and 40 μg/ml for all others; *i.e.*, the cells did not grow at concentrations greater than 10 μg/ml when the nonirradiated compound was used.

TABLE I. Inhibition of Growth of *E. coli* by Solutions of $(NH_4)_2(IrCl_6)$ Irradiated for Various Intervals in an Ammonia Environment Prior to Addition to the Culture Tubes.[a]

$(NH_4)_2(IrCl_6)$ (μg/ml)	% Inhibition after irradiation for 0 (hr):	1	2	3	5	7	24
10	17	3	7	7	10	4	10
20	100	2	7	8	10	15	17
30	100	4	7	6	18	17	21
40	100	10	11	15	21	15	31

[a] Values given are averages of 4 separate experiments, each of which was done in duplicate.

in the absence of an ammonia environment, no change whatsoever was detected. When exposed to the ammonia atmosphere in total darkness, the solutions slowly became virtually colorless, but did not undergo the further change to a violet color.

Electrophoresis of nonirradiated and irradiated solutions of $(NH_4)_2(IrCl_6)$ were consistent with the conversion of a strongly negative species, through a less strongly negative intermediate, to a product with no net electrical charge (Fig. 2). This observation thus verified the presence of an intermediate which was suggested by the spectral data, and was altogether analogous to the changes which are undergone by the corresponding platinum compound under closely similar conditions (1).

Biological effects of $(NH_4)_2(IrCl_6)$ on *E. coli* were strikingly dependent upon the duration of irradiation of the solutions prior to addition to the culture medium. Nonirradiated solutions were quite inhibitory (Table I), as was found by Rosenberg *et al.* (5), and those cells which were most resistant underwent cytokinesis (Fig. 3). After irradiation for 1 hr, virtually no inhibition of growth was evident, even at the highest concentration used. After irradiation for about 2 hr, the solutions still were not substantially inhibitory to growth as measured by optical densities of the cultures, but did induce formation of an appreciable number of filamentous forms. This inhibition of cytokinesis progressed with duration of irradiation until, at a 7-hr period of irradiation, the solutions when added to the culture medium resulted in the vast majority of the cell population being composed of enormously elongated forms (Fig. 4–8).

Interestingly, if the solution which was added to the cultures had been irradiated for 24 hr, the incidence of elongated forms was consistently less than that obtained at 7 hr. This finding may possibly be explained by analogy to another property of the corresponding platinum compound. The *cis* forms of certain neutral platinum chloroammino complexes in aqueous solution undergo isomerization to the *trans* configurations (7); the *trans* form of $[Pt(NH_3)_2Cl_2]^0$ is known to have no effect on *E. coli* (1). The neutral dichlorodiammino complex of palladium, another group VIII element, also rapidly undergoes such a *cis* to *trans* isomerization (8). If the neutral species finally produced by irradiation of aqueous solutions of $(NH_4)_2(IrCl_6)$ is indeed the *cis* isomer of tetrachlorodiammineiridium(IV), then such a similar conversion to the *trans* configuration may be the basis of the reduction of biological activity after 24 hr.

The foregoing thus indicates that another group VIII transition metal in the ionized state can undergo a photochemical reaction to a neutral form which selectively inhibits deoxyribonucleic acid synthesis in *E. coli* as reflected by inability of cells to divide while continuing to elongate. Unequivocally, extensive investigation of this compound as well as compounds of other elements in this group are essential to determine if these totally inorganic compounds may earn a rational role in chemotherapy of neoplasia.

Summary. When aqueous solutions of ammonium hexachloroiridate(IV) are irradiated with visible light in an ammonia atmosphere, a photochemical reaction occurs in which the complex $(IrCl_6)^{2-}$ ion is converted through a less negatively charged intermediate to an

apparently neutral species. Solutions so irradiated lose rather rapidly the absorption peaks at 416, 433, and 487 mμ and display a new peak at 325 mμ. Nonirradiated solutions are quite inhibitory to the growth of *Escherichia coli,* but irradiated solutions are much less so. Solutions irradiated for 5–7 hr markedly retard cytokinesis of the bacterial cells. It was tentatively proposed that the product responsible for this inhibition of cell division in the presence of continued cell elongation may be *cis*-tetrachlorodiammineiridium.

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