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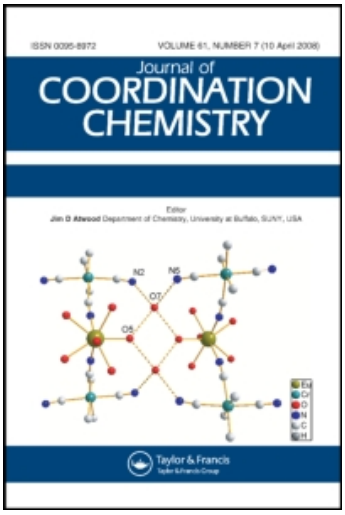

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STUDIES ON THE BACTERIAL ACTIVITY OF COBALT(III) COMPLEXES. PART III. COBALT(III) CARBOXYLATE COMPLEXES

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STUDIES ON THE BACTERIAL ACTIVITY OF COBALT(III) COMPLEXES. PART III. COBALT(III) CARBOXYLATE COMPLEXES

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Cobalt(III) complexes of the type $[Co(en)_2(chel)]X.nH_2O$ where en = ethylenediamine, chel = phthalato = $C_6H_4(CO_2)_2^{2-}$, maleato = $(O_2CCH=CHCO_2)^{2-}$, succinato = $(O_2CCH_2CH_2CO_2)^{2-}$, homophthalato = $(O_2CC_6H_4(CH_2)CO_2)^{2-}$, citraconato = $(O_2CC(CH_3)=CHCO_2)^{2-}$, itaconato = $(CH_2=C(CO_2)CH_2CO_2)^{2-}$, $X=NO_3^-, Br^-, (O_2CC_6H_4CO_2H)^-, (O_2CHC=CHCO_2H)^-, (O_2C(CH_2)_2CO_2H)^-, (O_2CC_6H_4(CH_2)CO_2H)^-$, $(O_2CHC=C(CH_2)\text{-}CO_2H)^-$, and $(O_2C\text{-}CH_2\text{-}C(=CH_2)\text{-}CO_2H)^-$, $[Co(en)_2(malonato)]X.2H_2O$ (where malonato = $(O_2CCH_2CO_2)^{2-}$, $X=Cl^-, Br^-$, and NO_3^-) and $[Co(en)_2CO_3]Cl.2H_2O$ have been investigated for their bacterial activity against *Escherichia coli B* growing on EMB agar and in minimal glucose media both in lag and log phases. Among the most active are where chel = phthalato and homophthalato. The effects are distinct from those known for compounds of Pt, e.g., *cis*-$[Pt(NH_3)_2Cl_2]$ and rhodium, e.g., *trans*-$[Rh(C_5H_5N)_4Cl_2].6H_2O$. Antagonisms are reported.

KEY WORDS: Co(III) complexes, dibasic aliphatic and aromatic acids, bacterial activity, phospholipids, antagonisms.

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INTRODUCTION

The toxicity of heavy metal compounds has been known for decades. It was found that cobalt(II), in relatively low concentrations, can prevent the growth of a large number of different species of bacteria in a medium otherwise capable of supporting vigorous growth.[1] The inhibition was found to be reversed by additions of histidine, with which cobalt(II) combines stoichiometrically. Some transition metal compounds including certain Co(III) species were reported to exhibit more selective biological activity.[2,3] It was observed that the shift in cellular chemistry induced by a particular complex appears to vary with the nature of the first coordination sphere.[4] The antibacterial activity of a number of Co(III) complexes of the general type $K[Co(Chel)_2(R\text{-}py)_2]H_2O$ was reported to be distinct from that of *cis*-platin and rhodium compounds.[5] Rhodium(II) carboxylates were reported to show a considerable

Throughout this paper, 1,2-benzenedicarboxylic acid, toluene-2-dicarboxylic acid, butanedioic acid, *cis*-butenedioic acid, propanedioic acid, methylmaleic acid and methylenesuccinic acid are called: phthalic acid, homophthalic acid, succinic acid, maleic acid, malonic acid, citraconic acid and itaconic acid, respectively.

variation in their antitumour activity against Ehrlich tumour cells in mice, with the butyrate complex being the most active.[6] Interest in Co(III) lies in the fact that it forms ligand complexes with donors such as ethylenediamine that are inert to substitution. The present work describes the antibacterial activity of a number of cobalt complexes of the general type $[Co(en)_2chel]X.nH_2O$, where chel are dibasic aliphatic and aromatic anions. Their inhibitory effects towards bacteria appear to be distinct from those of *cis*-platin, and rhodium(III) complexes previously examined.

EXPERIMENTAL

Elemental analyses were performed using a Carl Erba instrument. The turbidity of the growth culture was measured on an LKB Biochrom Ultrospec 4050 single beam spectrophotometer. Cultures were incubated in Gallenkamp orbital incubator at a temperature of $37° \pm 1°C$.

Preparation of the complex solution
The complexes were dissolved in distilled water, with heating whenever necessary. Solutions were filtered through a micromembrane filter (0.45 μm) aseptically into sterile tubes. Compound 3 was used as a slurry due to its insolubility in distilled water.

Determination of the zone of inhibition and MIC of the complexes on E. coli B *on EMB (eosin methylene blue) agar plates*
A well was aseptically bored in each plate after inoculation with a 24 h culture of *E. coli B*. Into the well (separate plates), different concentrations of the complexes were pipetted. The plates were incubated at 37°C for 24 h and the diameter of zone of inhibition was measured.

Determination of the effect of the complexes on E. coli B *in the minimal media, supplemented with glucose*
Into each minimal medium supplemented with glucose, 0.15 cm^3 of 16 h culture of a dilute minimal glucose culture (O.D. at 650 nm was 0.05–0.06) was innoculated. Four different concentrations of each complex were then pipetted into a separate flask and incubated at 37°C in a shaker incubator. The absorbance of the solution (turbidity) was determined every hour.

Lipid extraction from the yeast extract
About 20 g of yeast extract (Technical, Difco), was hydrolysed by addition of concentrated HCl. Then the lipid was extracted by the addition of diethylether which was then evaporated on steam bath. The product obtained was dissolved again in diethylether and evaporated to dryness, whereby a yellowish oil was obtained. The pH of the lipid was adjusted to neutrality or slight alkalinity with KOH (1 M) solution; the final concentration used was 25 $\mu g/cm^3$.

Determination of nutritional requirements and antagonistic effects with respect to growth inhibition of E. coli *by the complexes*
These studies were performed as previously described[17] except that the media were

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further supplemented with yeast extract (Technical, Difco, 0.3% w/v), casaamino acid (vitamin free, total N 7–11%, Difco, 0.1% w/v) and *L*-histidine (10^3 $\mu g/cm^3$). The media were sterilized separately at 100°C for 10 min (except for *L*-histidine which was filtered through a micromembrane filter) and then added to the minimal glucose media to make a final concentration of 0.3 and 0.1% of yeast extract and casaamino acid, respectively.

Antagonistic effect of phospholipid

These studies were carried out as described in the previous section, except that the media were supplemented with crude lipid from yeast extract without further purification and isolation of the individual components. These were tested against the inhibitory action of complexes 3, 4 and 8–13 ($5.57 \times 10^{-4} - 1.47 \times 10^{-3}$ M).

Synthesis of cobalt(III) complexes

The compounds (Table 1) for the most part were prepared by established methods.[7-9] However, $[Co(en)_2(itaconato)]X$ and $[Co(en)_2(citraconato)]X$, where $X = NO_3^-$ and Br^- were isolated for the first time; Duff[8] did not attempt to isolate these salts. Carbonatobis(ethylenediamine)cobalt(III) bromide dihydrate was prepared according to the literature.[9]

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$[Co(en)_2(itaconato)]Br.2H_2O$

Some 1.6 g (0.005 mol) of itaconic acid was added to a boiling solution of 5.0 g (0.016 mol) of carbonatobis(ethylenediamine)cobalt(III) bromide in 100 cm^3 of water. The solution, after being boiled for two minutes, was concentrated on a water bath and left in an ice chest. Yield: 88%.

$[Co(en)_2(itaconato)]NO_3.2H_2O$

This complex was obtained by reacting a solution of 3.00 g (0.007 mol) of $[Co(en)_2(itaconato)]Br.2H_2O$ in 50 cm^3 of water with 1.24 g of $AgNO_3$. After stirring for one hour, the silver bromide was collected and the filtrate evaporated on a waterbath. The nitrate separated slowly on cooling. Yield: 70.6%.

$[Co(en)_2(citraconato)]Br.2H_2O$

Some 1.6 g (0.005 mol) of citraconic acid was added to a boiling solution of 5 g (0.015 mol) of carbonatobis(ethylenediamine)cobalt(III) bromide in 100 cm^3 of water. The solution, after being boiled for two minutes was concentrated on a water bath and left in an ice chest. Yield: 94%.

$[Co(en)_2(citraconato)]NO_3.2H_2O$

This was obtained by reacting a solution of 3.0 g (0.007 mol) of $[Co(en)_2(citraconato)]$-$Br.2H_2O$ in 50 cm^3 of water with 1.24 g of $Ag(NO_3)$. After stirring for one hour, the silver bromide was collected and the filtrate evaporated on a water bath. The nitrate separated slowly on cooling. Yield: 91.6%.

Minimal medium supplemented with glucose

The minimal medium was prepared by dissolving dipotassium hydrogen orthophosphate

(10.50 g) in doubly distilled water. With stirring and low heating, potassium dihydrogen orthophosphate (4.50 g) was added and, after it had dissolved, trisodium citrate heptahydrate (0.47 g) and ammonium sulfate (1 g) were dissolved in this mixture. After cooling the mixture, magnesium sulfate heptahydrate (0.05 g) was added and the volume was made up to 1 dm^3 with distilled water. The pH of the solution was adjusted to 7.0. This minimal medium was placed in conical flasks, each containing 50.0 cm^3 and autoclaved at 121°C for 15 min. Then, 40% *D*.(+)-glucose solution was prepared and autoclaved separately; 1.25 cm^3 of the latter solution was added aseptically to the minimal medium.

RESULTS AND DISCUSSION

Twenty *bis*-ethylenediaminecarboxylatocobalt(III) salts and *bis*-ethylenediaminecarbonatocobalt(III) salts were prepared according to literature methods. Elemental analyses were satisfactory (Table 1).

Effect of the complexes on E. coli B *on EMB agar plates*

The results obtained on testing showed that the Co(III) complexes inhibited the growth of *E. coli B* on EMB agar (Tables 2 to 4). It is worth mentioning that inhibition is indicated by the clear area around the wells where the Co(III) complexes were injected. All complexes showed similar behaviour in their growth inhibition on the EMB agar. As observed in reported studies,[10-11] administration of a simple drop or more of solutions of the complexes does not much alter the diameter of the zone of inhibition. However, the zone of inhibition is much clearer when the complexes were administered to the well-culture plates.

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Table 1 Analytical data for the cobalt(III) dicarboxylate complexes[a]

b	a	C%	H%	N%
1	$[Co(en)_2(phthalato)]No_3.2.5H_2O$	33.7 (32.2)	5.2 (5.6)	15.6 (15.6)
2	$[Co(en)_2(phthalato)]Br.3H_2O$	30.3 (30.2)	4.7 (5.5)	12.1 (11.7)
3	$[Co(en)_2(phthalato)]O_2C.C_6H_4CO_2H.3H_2O$	42.9 (42.7)	4.8 (5.6)	9.7 (9.9)
4	$[Co(en)_2(maleato)]NO_3.H_2O$	25.9 (25.7)	5.6 (5.4)	19.3 (18.8)
5	$[Co(en)_2(maleato)]Br.2H_2O$	23.6 (23.5)	5.4 (5.4)	13.8 (13.7)
6	$[Co(en)_2(maleato)]O_2CCH=CH_2CO_2H.3H_2O$	31.2 (31.2)	5.5 (5.9)	11.5 (12.3)
7	$[Co(en)_2(succinato)]NO_3.H_2O$	24.3 (24.9)	5.4 (5.3)	18.4 (18.2)
8	$[Co(en)_2(succinato)]Br.2H_2O$	23.7 (23.6)	5.4 (5.3)	14.3 (14.2)
9	$[Co(en)_2(succinato)]O_2C(CH_2)_2CO_2H.4H_2O$	30.5 (30.0)	6.5 (6.2)	13.0 (12.8)
10	$[Co(en)_2(malonato)]Cl.2H_2O$	23.6 (23.6)	5.3 (5.2)	16.2 (16.2)
11	$[Co(en)_2(malonato)]Br.2H_2O$	22.2 (21.6)	4.8 (5.7)	14.4 (14.2)
12	$[Co(en)_2(malonato)]NO_3.2H_2O$	23.1 (22.7)	5.0 (5.9)	19.1 (18.9)
13	$[Co(en)_2CO_3]Cl.2H_2O$	21.5 (21.5)	6.0 (6.1)	21.1 (21.0)
14	$[Co(en)_2(homophthalato)]Br.H_2O$	34.1 (34.3)	5.3 (5.3)	12.6 (12.3)
15	$[Co(en)_2(homophthalato)]O_2CC_6H_4(CH_2)CO_2H.2H_2O$	46.2 (46.2)	4.8 (5.8)	9.6 (9.8)
16	$[Co(en)_2(citraconato)]Br.3H_2O$	24.4 (24.6)	5.0 (5.7)	13.3 (12.7)
17	$[Co(en)_2(citraconato)]NO_3.3H_2O$	25.3 (25.6)	5.7 (5.9)	16.9 (16.6)
18	$[Co(en)_2(citraconato)]O_2CC(CH_3)=CHCO_2H$	38.5 (38.5)	5.7 (5.1)	12.0 (12.8)
19	$[Co(en)_2(itaconato)]Br.3H_2O$	24.6 (24.5)	5.6 (5.9)	13.6 (12.7)
20	$[Co(en)_2(itaconato)]NO_3.3H_2O$	24.8 (25.5)	5.6 (6.2)	19.0 (16.5)

[a]Leaving ligand written last in formula[b]. Numbers for compounds are referred to throughout the text. Calculated figures are given in parentheses.

Table 2

a) Establishment of Minimum Inhibition Concentrations (MIC)

		Appearance of zone of inhibition[a] at the appropriate concentration (10^3 μg/plate)		
		25	50	100
1	$[Co(en)_2(phthalato)]NO_3.2.5H_2O$	+	+	+
2	$[Co(en)_2(phthalato)]Br.3H_2O$	+	+	+
3	$[Co(en)_2(phthalato)]O_2C.C_6H_4CO_2H.3H_2O$	+	+	+
4	$[Co(en)_2(maleato)]NO_3.H_2O$	+	+	+
5	$[Co(en)_2(maleato)]Br.2H_2O$	+	+	+
6	$[Co(en)_2(maleato)]O_2CCH=CH_2CO_2H.3H_2O$	+	+	+
7	$[Co(en)_2(succinato)]NO_3.H_2O$	+	+	+
8	$[Co(en)_2(succinato)]Br.2H_2O$	+	+	+
9	$[Co(en)_2(succinato)]O_2C(CH_2)_2CO_2H.4H_2O$	+	+	+
10	$[Co(en)_2(malonato)]Cl.2H_2O$	+	+	+
11	$[Co(en)_2(malonato)]Br.2H_2O$	+	+	+
12	$[Co(en)_2(malonato)]NO_3.2H_2O$	+	+	+
13	$[Co(en)_2CO_3]Cl.2H_2O$	+	+	+

[a] + = inhibition.

b) Inhibition by Cobalt(III) Complexes

		Diameter of zone of inhibition (CM) at various concentrations (10^3 μg/plate)		
		25	50	100
1	$[Co(en)_2(phthalato)]NO_3.2.5H_2O$	2.65	2.6	2.75
2	$[Co(en)_2(phthalato)]Br.3H_2O$	2.4	2.4	2.4
4	$[Co(en)_2(maleato)]NO_3.H_2O$	2.3	2.3	2.3
5	$[Co(en)_2(maleato)]Br.2H_2O$	2.4	2.7	3.2
6	$[Co(en)_2(maleato)]O_2CCH=CH_2CO_2H.3H_2O$	2.4.	3.5	2.8
7	$[Co(en)_2(succinato)]NO_3.H_2O$	2.3	2.6	3.5
8	$[Co(en)_2(succinato)]Br.2H_2O$	2.3	2.5	2.8
9	$[Co(en)_2(succinato)]O_2C(CH_2)_2CO_2H.4H_2O$	2.3	4.0	4.0
10	$[Co(en)_2(malonato)]Cl.2H_2O$	2.2	2.2	2.2
11	$[Co(en)_2(malonato)]Br.2H_2O$	1.2	2.1	2.2
12	$[Co(en)_2(malonato)]NO_3.2H_2O$	2.4	2.8	2.9
13	$[Co(en)_2CO_3]Cl.2H_2O$	0.5	1.2	1.2

Extension of the lag phase and its apparent effect on growth

The growth of *E. coli B* in minimal media supplemented with glucose in the presence of Co(III) complexes shows that compounds 1, 3, 6–15, 19 and 20 are toxic at an average of $5.1 \times 10^{-4} - 2.18 \times 10^{-3}$ M. Compounds 2, 4, 5, 16 and 17 inhibit growth (extended lag phase) at an average of $5.0 \times 10^{-4} - 1.6 \times 10^{-3}$ M and are toxic at concentrations $> 2.1 \times 10^{-3}$ M. Initial inhibition of growth was apparently exerted during the early stages of growth by extension of the lag phase; afterwards, reduction of overall growth took place in the log phase (the slope of the plot of optical density *versus* time decreased). This effect (extent of lag), depended on the nature of the complexes; the higher concentrations used are lethal to the bacteria as shown in Figures 1A–F, 2–4A. From Figure 1F, 2A, 3A and 4A, it is obvious that complexes

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Table 3 Establishment of minimum inhibitory concentration (MIC) values as determined by conventional EMB agar dilution procedure

		Variable colonies count (10^4) at the appropriate concentration (10^3 μg/plate)						
		Control	5	12.5	25	37.5	50	62.5	75
		333							
2	$[Co(en)_2(phthalato)]Br.3H_2O$		166	108	0	0	0	0	0
4	$[Co(en)_2(maleato)]NO_3.H_2O$		0	0	0	0	0	0	0
5	$[Co(en)_2(maleato)]Br.2H_2O$		0	0	0	0	0	0	0
6	$[Co(en)_2(maleato)]O_2CCH=CH_2CO_2H.3H_2O$		96	11	0	0	0	0	0
7	$[Co(en)_2(succinato)]NO_3.H_2O$		135	73	21	23	0	0	0
8	$[Co(en)_2(succinato)]Br.2H_2O$		132	82	21	0	0	0	0
9	$[Co(en)_2(succinato)]O_2C(CH_2)_2CO_2H.4H_2O$		140	85	0	0	0	0	0
11	$[Co(en)_2(malonato)]Br.2H_2O$		200	100	32	22	0	0	0
13	$[Co(en)_2CO_3]Cl.2H_2O$		116	96	0	0	0	0	0
14	$[Co(en)_2(homophthalato)]Br.H_2O$		165	139	101	28	29	0	0
15	$[Co(en)_2(homophthalato)]O_2CC_6H_4(CH_2)CO_2H.2H_2O$		266	149	150	82	56	0	0
16	$[Co(en)_2(citraconato)]Br.3H_2O$		90	61	80	28	0	0	0
17	$[Co(en)_2(citraconato)]NO_3.3H_2O$		134	65	83	80	0	0	0
18	$[Co(en)_2(citraconato)]O_2CC(CH_3)=CHCO_2H$		157	95	74	0	0	0	0

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Table 4 Establishment of minimum inhibitory concentration (MIC) values as determined by counts of viable bacterial cells/ml following overnight incubation in minimal glucose media

		Viable bacterial cells/ml ($\times 10^6$) at the appropriate concentration ($\times 10^2$ μg/ml)											
		Control		2.00		4.00		6.00		8.00		10.00	
		Z[a]	E[a]	Z	E	Z	E	Z	E	Z	E	Z	E
1	$[Co(en)_2(phthalato)]NO_3.2.5H_2O$	0.8	837.6	1.6	5.6	4.8	6.4	0.8	8	9.6	7.2	[b]	[b]
2	$[Co(en)_2(phthalato)]Br.3H_2O$	0.8	837.6	2.4	518.4	5.6	536.8	3.2	56	5.6	14.9		
3	$[Co(en)_2(phthalato)]O_2C.C_6H_4CO_2H.3H_2O$	12.8	920.8	20.8	18.4	29.6	24.8	31.2	24.8	54.4	46.4		
4	$[Co(en)_2(maleato)]NO_3.H_2O$	13.6	800.8	12.8	536	8	7.2	10.4	9.6	12.8	13.6		
5	$[Co(en)_2(maleato)]Br.2H_2O$	13.6	800.8	13.6	604	11.2	382.4	8.8	252	10.4	39.2		
6	$[Co(en)_2(maleato)]O_2CCH=CH_2CO_2H.3H_2O$	13.6	800.8	5.6	8.8	12.8	131	12.8	136	12.0	12.0		
7	$[Co(en)_2(succinato)]NO_3.H_2O$	13.6	800.0	11.2	8.0	7.2	9.6	15.2	14.4	16.0	13.6		
8	$[Co(en)_2(succinato)]Br.2H_2O$	13.6	800.8	10.4	711.2	9.6	6.4	8.0	9.6	16.0	12.0		
9	$[Co(en)_2(succinato)]O_2C(CH_2)_2CO_2H.4H_2O$	13.6	800.8	11.2	12.8	9.6	6.4	11.2	11.2	12.0	8.8		
10	$[Co(en)_2(malonato)]Cl.2H_2O$	12.8	920.8	4.8	7.2	7.2	10.4	12.0	16.0	16.0	16.8		
11	$[Co(en)_2(malonato)]Br.2H_2O$	12.8	920.8	13.6	13.6	11.2	6.4	12.8	12.0	11.2	16.8		
12	$[Co(en)_2(malonato)]NO_3.2H_2O$	12.8	920.8	16.0	260.8								
13	$[Co(en)_2CO_3]Cl.2H_2O$	12.8	920.8	8.0	15.2	16.0	19.2	18.4	20.0	17.6	16.0		
14	$[Co(en)_2(homophthalato)]Br.2H_2O$	43.2	1028.0	40.8	32.8	48.8	46.4	59.2	51.2	64.0	60.8	85.6	75.2
15	$[Co(en)_2(homophthalato)]O_2CC_6H_4(CH_2)CO_2H.2H_2O$	43.2	1028.0	36.8	34.4	34.4	35.2	47.2	45.6	54.4	66.4	56.8	58.4
16	$[Co(en)_2(citraconato)]Br.3H_2O$	43.2	1028.0	181.6	942.4	142.4	850.4	120.0	672.0	111.2	291.2	97.6	128.0
17	$[Co(en)_2(citraconato)]NO_3.3H_2O$	43.2	1028.0	190.4	1052.8	160.8	964.8	138.4	764.8	131.2	257.6	128.0	178.4
18	$[Co(en)_2(citraconato)]O_2CC(CH_3)=CHCO_2H$	43.2	1028.0	219.2	1018.4	229.6	978.4	209.6	957.6	209.6	953.6	214.4	929.6
19	$[Co(en)_2(itaconato)]Br.3H_2O$	36.8	1035.2	43.2	23.2	41.6	37.6	56.8	52.8	56.4	44.8	61.6	52.0
20	$[Co(en)_2(itaconato)]NO_3.3H_2O$	44.0	1080.8	56.0	236.8	51.2	42.4	62.4	37.6	64.8	57.6	70.4	59.2
21	$[Co(en)_2(itaconato)]CH_2=C(CO_2)CH_2CO_2H$	44.0	1080.8	55.2	1016.8	56.8	1020.8	49.6	966.6	59.2	956.0	59.2	753.6

[a]Z = zero time, E = end of incubation period. [b]Not tested at these conditions, where no figures are given.

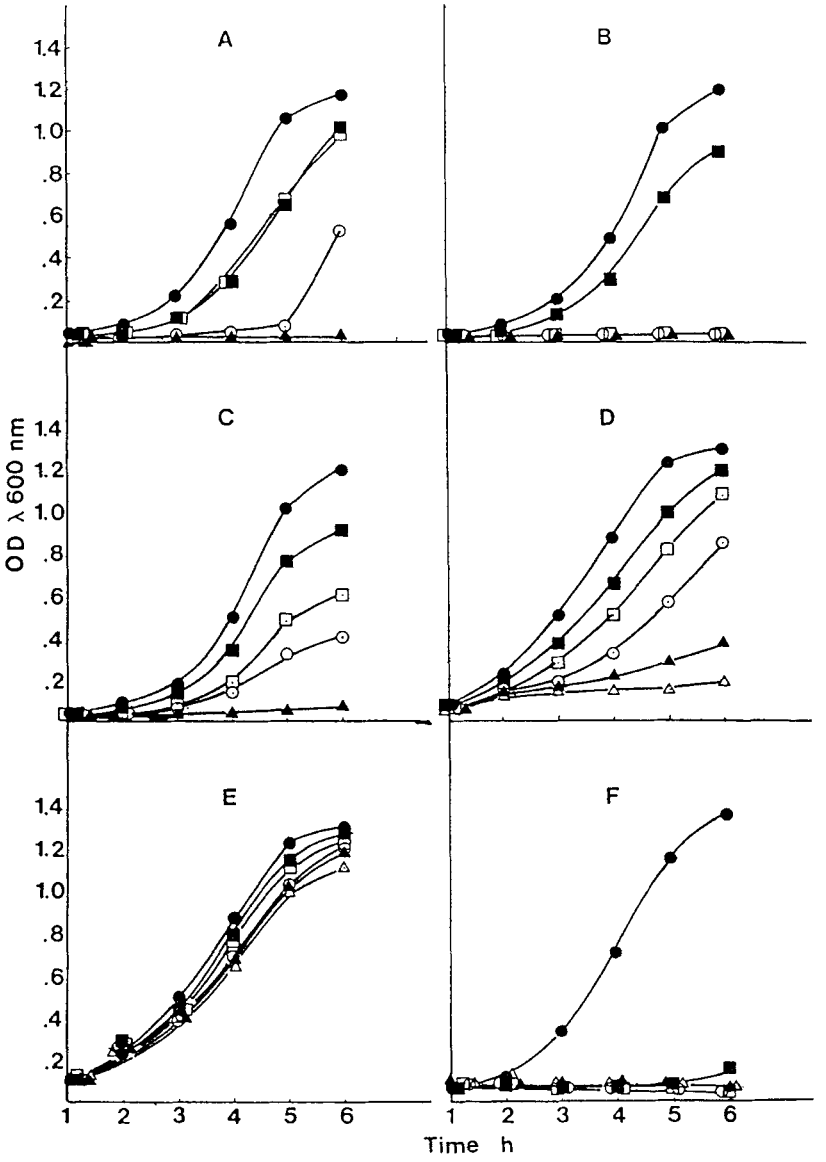

Figure 1 The effect of cobalt complexes added prior to the bacterial inoculums on the growth of *E. coli B* in aerated glucose minimal medium at 37°C and pH 7.0. Compound numbers refer to Table 1; A) compound 2: (●——●) control; (■——■) 4.78×10^{-4} M; (□——□) 9.56×10^{-4} M; (○——○) 1.43×10^{-3} M; (▲——▲) 1.91×10^{-3} M; B) compound 4: (●——●) control; (■——■) 5.37×10^{-4} M; (□——□) 1.07×10^{-3} M; (○——○) 1.61×10^{-3} M; (▲——▲) 2.15×10^{-3} M; C) compound 5: (●——●) control; (■——■) 4.89×10^{-4} M; (□——□) 9.78×10^{-4} M; (○——○) 1.46×10^{-3} M; (▲——▲) 1.95×10^{-3} M; D) compound 16: (●——●) control; (■——■) 5.16×10^{-4} M; (□——□) 1.03×10^{-3} M; (○——○) 1.55×10^{-3} M; (▲——▲) 2.06×10^{-3} M; (△——△) 2.50×10^{-3} M; E) compound 18: (●——●) control; (■——■) 4.58×10^{-4} M; (□——□) 9.16×10^{-4} M; (○——○) 1.38×10^{-3} M; (▲——▲) 1.83×10^{-3} M; (△——△) 2.29×10^{-3} M; F) compound 20: (●——●) control; (■——■) 2.72×10^{-5} M; (□——□) 1.03×10^{-3} M; (○——○) 1.6×10^{-3} M; (▲——▲) 2.17×10^{-3} M; (△——△) 2.72×10^{-3} M.

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20, 14, 10 and 19 show complete inhibition of bacterial growth compared to the growth curve for the control. At sub-lethal concentrations, the cells grow but do so more slowly than controls, thus indicating that the complexes extend the lag phase of growth. This was particularly evident for complexes 2, 4, 5, 16 and 18 (Figure 1A–E). However, complexes 2, 4, 5, 16 and 18 showed variation of inhibition in the order $4>2>5>16>18$. Inhibition of bacterial growth decreases in the order 1, 3, 6, 7, 9, $10>11>12>15>19>20>4>8>5>16>17>18>21$.

The growth inhibition pattern (Figure 1A–F) also varies with the bidentate chelate: phthalate $\approx$ homophthalate > succinate > malonate > maleate > itaconate > citraconate, as well as the counter anions. The latter order was $NO_3^- \approx$ phthalate$^- > Br^-$ with respect to phthalato complex; $NO_3^- \approx$ succinato $> Br^-$ with respect to succinato; maleate$^- > NO_3^- > Br^-$ with respect to maleato; Cl^-, $\approx Br^- > NO_3^-$ with respect to malonate; $Br^- \approx$ homophthalate$^-$ with respect to homophthalato; $Br^- > NO_3^-$ > citraconate$^-$ with respect to citraconato and $Br^- > NO_3^-$ > itaconate$^-$ with respect to itaconato complex.

In any biological system, growth may be defined as the orderly increase of all chemical components. In an adequate medium, to which they have become fully adapted, bacteria are in a state of balanced growth. The addition of complexes of Co(III) to the growing culture of *Escherichia coli B* just entering the log phase was made to ascertain whether these complexes have any effect on the growth (in comparison to our previous studies where Co(III) complexes were added during the lag phase). The injection of aqueous complexes 4, 10, 11, 12, 15, 17, 18 and 21 had no effect on rate of growth. Representative of these complexes is complex 10 (Figure 3). Addition of the complexes 1, 3, 6, 9, 14, 19 and 20 halted growth after one generation as shown in Figures 2 and 4. Samples taken after such addition did not show (under the microscope) any gradual increase in the incidence of filamentation of cells as compared with effects of rhodium(III) *S*-nicotine complexes.[12]

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The antagonistic effect of nutrients on the growth inhibition of Escherichia coli B

Earlier, it was shown that growth inhibition was apparently exerted during the early stages of growth. At sub-lethal concentrations, cells grow (but more slowly than the controls); the Co(III) compounds thus extended the lag phase of growth. The length of the lag period can be reduced in many cases by addition of a variety of nutrients: yeast extract, casaamino acid (vitamin free), three groups of different amino acids (such as group one: *L*-alanine, asparagine, arginine hydrochloride, *L*-methionine, *L*-threonine, *L*-valine, *L*-phenylalanine, *L*-serine, *L*-proline, hydroxyproline and glycine dissolved in water; group two: cystine and tryptophane dissolved in 0.2 M HCl; group three: aspartic acid and glutamic acid dissolved in 0.2 M NaOH). Water soluble vitamin mixtures (inositol, pantothenic acid, calcium salts and biotin) and vitamin B_1 (aneurine HCl, thiamine HCl), and *L*-histidine commonly have such an effect on a wide range of bacteria and especially yeast extract which supplies a variety of factors including purines, pyrimidines, amino acids, peptides and vitamins.

This study was carried out to determine the effect of nutrients on the inhibitory action of Co(III) complexes in light of the previous lag phase. The effect of complexes 1–4, 6–15, 19 and 20 ($5.1\times10^{-4}-2.1\pm10^{-3}$ M) on the growth of *E. coli B* was determined in minimal glucose media to which was added, respectively, yeast extract (0.3% w/v), casaamino acid (0.1% w/v vitamin free), different groups of amino acids, vitamin mixture and vitamin B1 (20 μg/cm^3 and 10 μg/cm^3, respectively), and *L*-histidine (10^3 μg/cm^3).

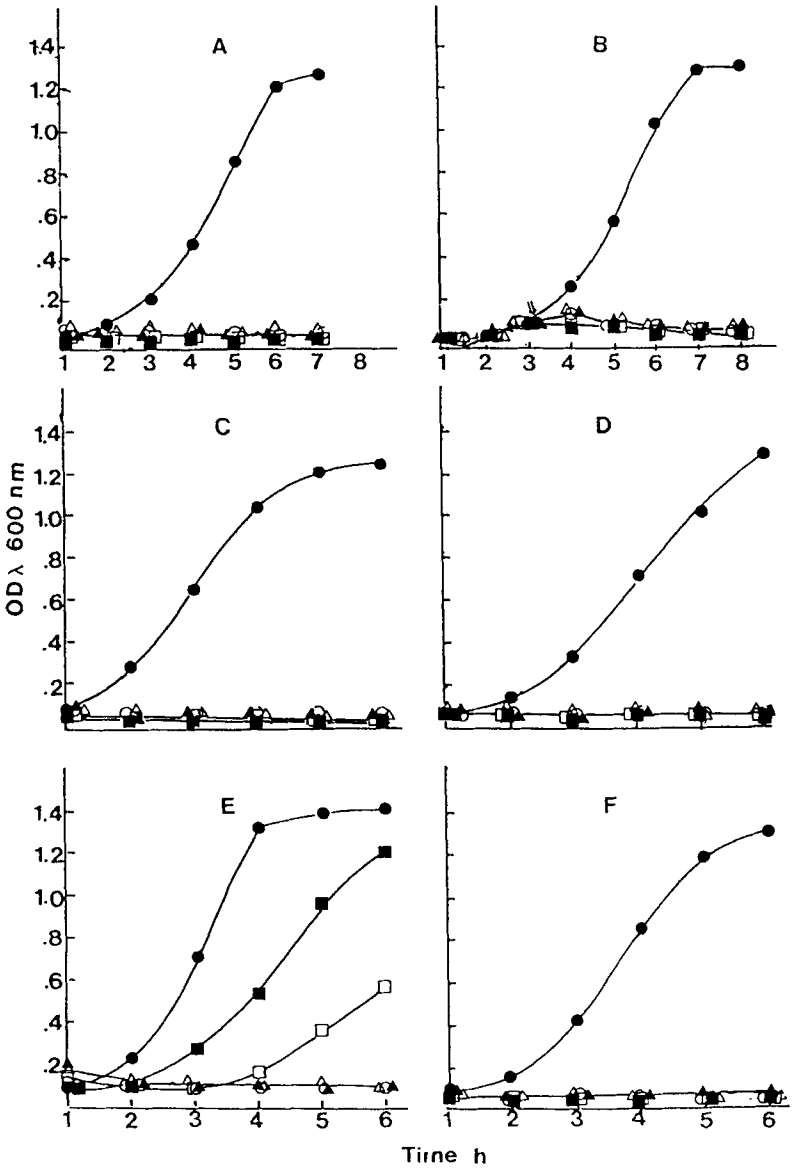

Figure 2 The effect of compound 14 added prior to the bacterial inoculums on the growth of *E. coli B* in aerated glucose minimal medium at 37°C and pH 7.0; A) lag phase; B) log phase (as indicated by arrow; C) in the presence of casaamino acid (0.1% w/v); D) in the presence of group 1–3 amino acids; E) in the presence of yeast extract (0.3% w/v); F) in the presence of 20 $\mu g/cm^3$ of lipid extracted from yeast extract; (●——●) control; (■——■) 4.63×10^{-4} M; (□——□) 9.25×10^{-4} M; (○——○) 1.38×10^{-3} M; (▲——▲) 1.85×10^{-3} M; (△——△) 2.31×10^{-3} M.

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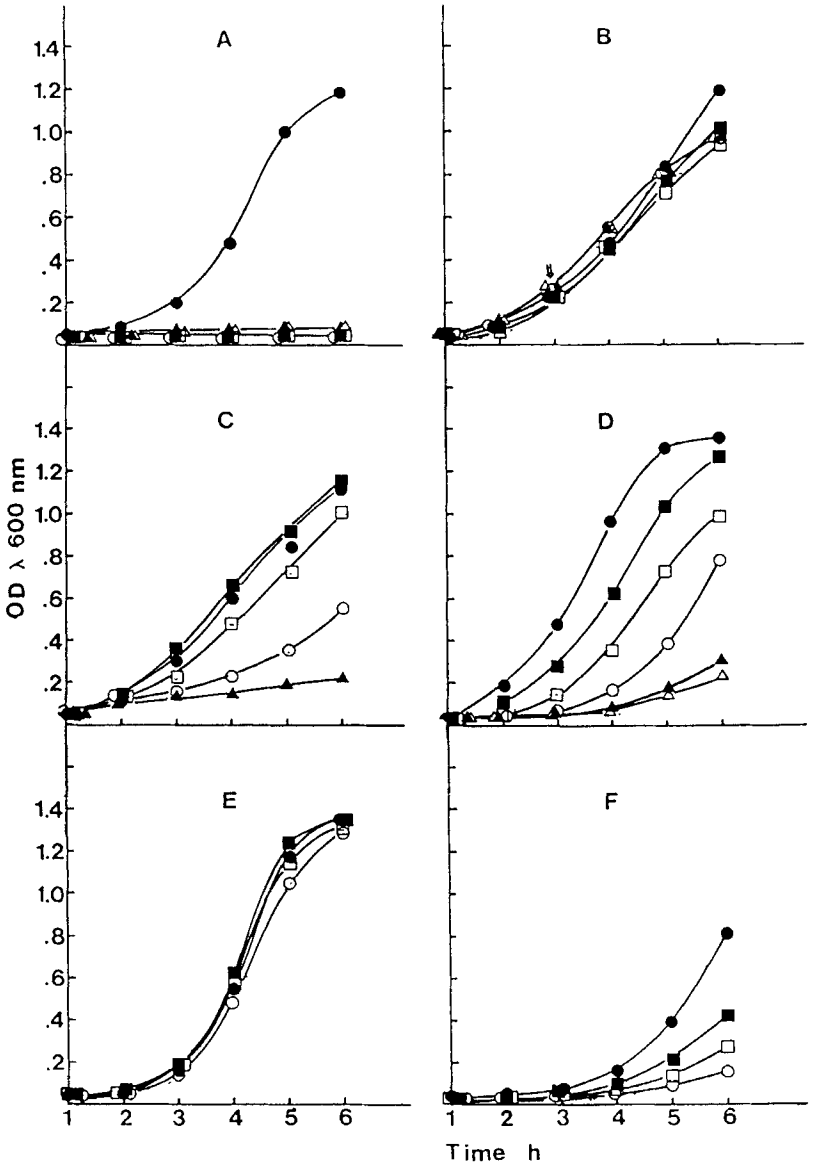

Figure 3 The effect of compound 10 added prior to the bacterial inoculum on the growth of *E. coli B* in aerated glucose minimal medium at 37°C and pH 7.0; A) lag phase; B) log phase as indicated by arrow; C) in the presence of casaamino acid (0.1% w/v); D) in the presence of group 1 amino acids (20 $\mu g/cm^3$); E) in the presence of (0.3% w/v) yeast extract; F) in the presence of (20 $\mu g/cm^3$) lipid extracted from yeast extract; (●——●) control; (■——■) 2.67×10^{-4} M; (□——□) 1.13×10^{-3} M; (○——○) 1.70×10^{-3} M; (▲——▲) 2.27×10^{-3} M; (△——△) 2.83×10^{-3} M.

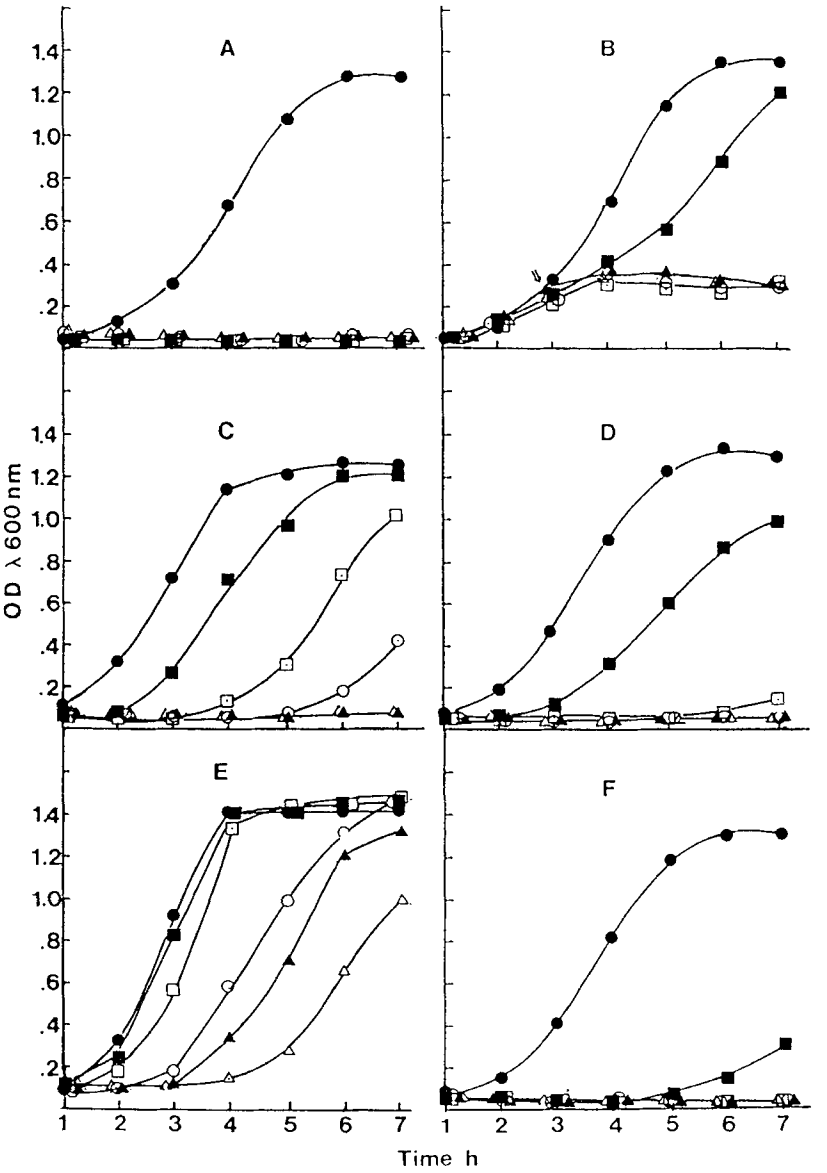

Figure 4 The effect of compound 19 added prior to the bacterial inoculums on the growth of *E. coli B* in aerated glucose minimal medium at 37°C and pH 7.0; A) lag phase; B) log phase as indicated by arrow; C) in the presence of casaamino acid (0.1% w/v); D) in the presence of group 2 amino acids (20 $\mu g/cm^3$); E) in the presence of (0.3% w/v) yeast extract; F) in the presence of (20 $\mu g/cm^3$) lipid extracted from yeast extract; (●——●) control; (■——■) 5.18×10^{-4} M; (□——□) 1.04×10^{-3} M; (○——○) 1.55×10^{-3} M; (▲——▲) 2.07×10^{-3} M; (△——△) 2.59×10^{-3} M.

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From measurements of turbidity at 600 nm when 0.3% w/v of yeast extract was added, the inhibitory actions ($5.57 \times 10^{-4} - 1.47 \times 10^{-3}$ M) of complexes 2, 4, 6–13, 15, 19 and 20 are antagonised effectively whereas complex 14 is only affected at lower concentrations ($4.63 \times 10^{-4} - 9.25 \times 10^{-4}$ M). However, in the case of complexes 1 and 3, the inhibition of the growth of *E. coli B* persisted, even when casaamino acids (0.1% w/v) were added. Thus it is likely that a component in the yeast extract is providing the effect or is reacting with the complexes so as to modify inhibitory effects.

In this work, most of the complexes which showed inhibition effects were antagonised by 0.3% w/v yeast extract (Figures 2–4E show effects of some complexes); e.g., compound 1 is affected at $4.78 \times 10^{-4} - 9.56 \times 10^{-4}$ M only (above this range inhibition persisted, and similarly for compound 3). However, when casaamino acid (0.1% w/v) was added to the minimal glucose media to which compounds 2, 4, 6–15, 19 and 20 were present at concentrations of $5.57 \times 10^{-4} - 1.47 \times 10^{-3}$ M, only compounds 15, 19 and 20 showed little response towards antagonism by casaamino acids, whereas compounds 10–12 responded well (Figures 2–4C represent some of the compounds). It was thus concluded that casaamino acid provided a variety of different amino acids which contributed to its slight antagonism against those complexes which showed a response to the presence of such amino acids. In order to find out which group of amino acids had caused such antagonism, three different groups were tested. Compounds 2, 4, 6–15, 19 and 20 were added ($5.57 \times 10^{-4} - 1.47 \times 10^{-3}$ M) to minimal glucose media to which the three different groups of amino acids were added separately at concentrations of 20 $\mu g/cm^3$. Only compounds 10–12, 15 and 19 showed response to the presence of these amino acids, especially group two; only compounds 10–12 responded to group one (Figures 2–4D show representative examples). Vitamin mixture and *L*-histidine HCl at 10 $\mu g/cm^3$ and 10^3 $\mu g/cm^3$, respectively, showed no antagonism towards the inhibitory action of Co(III) complexes.

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The antagonistic effect of phospholipids extracted from yeast extract

The addition of yeast extract (0.3%) completely removed the inhibition caused by all the Co(III) carboxylate complexes present in a concentration above that causing complete inhibition. For the lower concentrations, yeast extract reversed the extension of lag phase. None of the other nutrients present in yeast (vitamins, amino acids) had any effect except that some groups of amino acids antagonised the inhibitory effect of Co(III) complexes. Since the main water-soluble components of yeast extract had no effect, the lipid fraction was separated. The antagonistic effect of lipid extracted from yeast extract on the growth inhibition of *E. coli B* was investigated by adding 25 $\mu g/cm^3$ of lipid extracted from yeast extract to the minimal glucose media into which Co(III) complexes 3–14, 19 and 20 were added at $5.57 \times 10^{-4} - 1.47 \times 10^{-3}$ M.

The antagonistic effect of the phospholipid extract was not exhibited for complexes 3, 4, 6–9, 15–19 and 20 whereas compounds 10–13 were antagonised. The lipids also increased the minimum inhibitory concentration (MIC) needed for complete inhibition. Figures 2–4E/F show how some compounds are effected by yeast extract as compared to the lipid extract, where the lag phase was shortened, and growth rate increased.

One may view the potential of these studies in several ways. First, interest in these Co(III) complexes lies in the fact that are very inert to substitution often. This means that activity may be related to the hydrolysis of the carboxylate anions. Such a process[13–16] might generate active species from the complexes $[Co(en)_2(chel)]X$.

Secondly, molecular reorganisation leading to growth inhibition must be considered a possibility as it is observed that all complexes are active in the lag phase and some in the log phase of growth (Figures 1A–F and 2–4B).

The length of lag period can be reduced in many cases by the addition of variety of nutrients. Amino acids, purines and pyrimidines commonly have such an effect on a wide range of bacteria but had no marked effect in this work, nor did they reduce the extension of lag caused by the cobalt complexes. The antagonistic effect of yeast extract was due to its lipid content. Lipid extracts had no effect on the lag phase of uninhibited controls, only on the extension of this by the cobalt complexes. Lipids also increased the minimum inhibitory concentration needed for complete inhibition. These results supported a previous discovery,[5] in which other Co(III) complexes of the type $K[Co(chel)(py)_2]$, where chel $= CO_3^{2-}$, $C_2O_4^{2-}$, $C_2H_2O_4^{2-}$ had been tested against *E. coli B*. Their inhibitory effect was also reversed by lipid. These complimentary results and others,[5,17] are not yet adequate to locate the mechanism of inhibition of early growth. It is, however, tempting to suggest that the cobalt complexes inhibit the formation of a membrane lipid by interfering either with its synthesis or its incorporation into a membrane organell before rapid and exponential growth is assumed. The synthesis of cell membranes, particularly the cytoplasmic membrane, is essential if cells are to reproduce normally at their maximum rates.

In the present case, the cobalt compounds $[Co(chel)(en)_2]X.H_2O$ clearly interfere earlier in the cell division and growth cycles than do salts of the rhodium compounds $[Rh(S\text{-nicotine})_4X_2]^+$, which in turn seem to act earlier than platinum compounds of the type *cis*-$[Pt(NH_3)_2X_2]$.

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