Effect of Chloramphenicol on Ribonucleic Acid Synthesis in Liver Cells in Suspension

By S. T. JACOB and P. M. BHARGAVA
Regional Research Laboratory, Hyderabad 9, India

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1. Chloramphenicol has a stimulatory effect on the incorporation of radioactive phosphate into the RNA of perfused rat-liver slices, whole liver homogenates or the liver-cell suspensions, and no effect on the incorporation of [^{14}C]adenine and [^{14}C]uracil into the RNA of the tissue slices. 2. Chloramphenicol completely inhibits the incorporation of labelled adenine and uracil into the RNA of the cell suspensions, or into the RNA of homogenates derived from the whole liver tissues. 3. Chloramphenicol has at most a slight inhibitory effect on the transport of labelled adenine or uracil in the hepatic cells in suspension; in the slices, the transport of these bases is not inhibited at all. 4. The above observations indicate that: (*a*) unlike the tissue slices, hepatic cells in suspension are permeable to chloramphenicol; (*b*) in the presence of chloramphenicol, for reasons that are not clear, the conversion of the base into the appropriate nucleotide does not proceed.

The effect of chloramphenicol on RNA synthesis has been studied by many investigators (see the Discussion section for references). These studies have been confined to bacteria (growing cell population, resting cells or protoplasts), except for two investigations, one on calf-thymus nuclei (Breitman & Webster, 1958) and the other on ascites-tumour cells (LePage, 1953). Further, in most of these reports the criterion of RNA synthesis has been either net increase in the amount of RNA, or the incorporation of [^{32}P]phosphate or [^{14}C]uridine into RNA; in none of these reports was the synthesis of RNA found to be inhibited (in fact, in some cases, a slight stimulation was observed) by chloramphenicol when used at concentrations up to 1000μg./ml. Only in two investigations (Mandel & Altman, 1961; Kapoor, Sagar & Agarwala, 1963), both on bacteria, was the effect of chloramphenicol on the incorporation of a free base into RNA studied; this incorporation was found to be inhibited by low concentrations (10–100μg./ml.) of the antibiotic.

In a previous paper (Jacob & Bhargava, 1965) we have shown that rat-liver cells obtained in suspension by the method of Jacob & Bhargava (1962) can incorporate labelled adenine, uracil and phosphate into their RNA; liver-cell suspensions prepared by this method have also been shown to respire (Iype & Bhargava, 1965) and synthesize protein (Bhargava & Bhargava, 1962). In the present studies it is shown that in rat-liver-cell suspensions, unlike in the liver tissue slices, the incorporation of labelled adenine or uracil into RNA is completely inhibited by low concentrations (50μg./ml.) of chloramphenicol. However, the incorporation of labelled phosphate into RNA is not inhibited by chloramphenicol, either in the tissue slices or in the cell suspensions. These observations are discussed *vis-à-vis* the previous observations, mentioned above, on the effect of chloramphenicol on RNA synthesis.

EXPERIMENTAL

Materials

Animals. Adult Wistar albino rats, weighing more than 150g. and fed *ad libitum*, were used; the rats were of an inbred strain from the Nutrition Research Laboratory, Hyderabad. In any one experiment, the perfused tissue slices and the liver-cell suspensions were obtained from the same animal.

Radioactive chemicals. [^{32}P]Phosphate was obtained from the Atomic Energy Establishment, Bombay, as orthophosphate in 0·9% NaCl buffered with phosphate buffer to give a concentration of 1mg. of P/ml. (specific radioactivity 1mc/mg.). [8-^{14}C]Adenine and [2-^{14}C]uracil were obtained from the Atomic Energy Establishment, Bombay, and The Radiochemical Centre, Amersham, Bucks., and dissolved in 0·9% NaCl to give a concentration of 9·8μmoles (9·0μc)/ml. and 0·9μmole (9·1μc)/ml. respectively.

Buffers. The tissue preparations were incubated in either Krebs–Ringer bicarbonate buffer or Ca^{2+}-free Krebs–Ringer phosphate buffer (Dawson, Elliott, Elliott & Jones, 1959).

Chloramphenicol. Chloramphenicol (Chloromycetin, a gift from Parke, Davis and Co., Bombay) was dissolved in 0·9% NaCl to give a stock solution containing 1mg./ml.

Methods

Preparation of liver slices. Slices (500–600 μ thick) were cut free-hand with a razor.

Preparation of liver-cell suspensions. The cell suspensions were prepared by the method of Jacob & Bhargava (1962).

Preparation of homogenates. The homogenates were prepared in the cold in 10 vol. of Krebs–Ringer phosphate buffer by using a Potter–Elvehjem homogenizer.

Incubation. The slices [50–150 mg. wet wt. (approx. 10–30 mg. dry wt.)/ml. of the medium] and the liver-cell suspension [containing 6×10^6–25×10^6 hepatic cells (having a dry wt. of approx. 9–38 mg.)/ml.] were incubated in the desired buffer with the radioactive precursor in 25 ml. conical flasks at 37°, in a constant-temperature bath with shaking at 100 oscillations/min. All the incubations were commenced within 30 min. of killing the animal. Separate 0 hr. samples were run for each tissue preparation.

Preparation of subcellular fractions. These were obtained from the slices of the cell suspension after incubation with the radioactive precursor. The tissue preparation was washed several times with cold Ca^{2+}-free Krebs–Ringer phosphate buffer until the washings were free of radioactivity; it was then suspended in about 10 ml. of cold 0·25 M-sucrose and homogenized for 2 min. in the cold by using a Potter–Elvehjem homogenizer. The nuclear fraction was sedimented by centrifugation at 4° for 10 min. at 700*g* in an MSE refrigerated centrifuge; the sediment was resuspended in 0·25 M-sucrose and centrifuged again as above. The combined supernatants were centrifuged for 10 min. at 10000*g* in a Spinco model L ultracentrifuge to sediment the mitochondria, which were resuspended in 0·25 M-sucrose and sedimented again as above. The microsomal fraction was sedimented from the combined supernatants by centrifugation at 105000*g* for 60 min. and washed once. The combined supernatants obtained at this stage are referred to as the 'supernatant' fraction of the cell. All operations were carried out quantitatively.

Isolation of the total nucleic acids. The total nucleic acid fraction was obtained by extraction with 10% (w/v) NaCl of the trichloroacetic acid precipitate of the tissue preparation or the subcellular fraction, either after removal (method I) or before removal (method II) of the lipids; the details are described by Jacob & Bhargava (1965).

Preparation of the acid-soluble fraction. The combined supernatants obtained after removal and washing of the trichloroacetic acid precipitate were repeatedly extracted with ether to remove trichloroacetic acid, and the aqueous phases were neutralized to pH 7.

Separation of the RNA fraction from the total nucleic acids. RNA was obtained in the hydrolysed form by treatment of the total nucleic acid fraction with 0·3 N-KOH at 37° for 18 hr., followed by adjustment of the pH to 4 in the cold with 0·6 N-$HClO_4$ and removal of the DNA–$KClO_4$ precipitate.

Separation of nucleotides. The ribonucleotides in the alkaline hydrolysate obtained as above were separated by paper electrophoresis, eluted and estimated as described by Davidson & Smellie (1952).

Estimation of nucleic acids. An extinction value of 1·0 in the neutralized RNA fraction obtained on alkaline hydrolysis of the total nucleic acids was taken to correspond to 33 μg. of RNA/ml.

Measurement of radioactivity. A known volume of a solution containing the radioactive material was plated on aluminium planchets (2 $cm.^2$), which were counted in either a Philips or an Ekco end-window counter, each with 6 and 10% counting efficiency for ^{14}C and ^{32}P respectively. All the samples were counted to a statistical significance of 5% as recommended by Calvin, Heidelberger, Reid, Tolbert & Yankwich (1949). Appropriate correction for self-absorption was made for ^{14}C-containing samples where

Table 1. *Effect of chloramphenicol on the incorporation of* [^{14}C]*adenine into the RNA of rat-liver slices and cell suspensions*

Incubations were carried out in 4 ml. of Ca^{2+}-free Krebs–Ringer phosphate buffer. The concentrations of chloramphenicol and [^{14}C]adenine were 50 μg./ml. and 0·49 μmole (0·45 μc)/ml. respectively. The total nucleic acid fraction (RNA + DNA), from which RNA was obtained in the hydrolysed form, was isolated by method I (see the text). Values in parentheses give the percentage changes in the specific activity of RNA with chloramphenicol.

		Specific activity of RNA (counts/min./mg.)			
		Cell suspensions		Perfused liver slices	
Expt. no.	Period of incubation (min.)	Without chloramphenicol	With chloramphenicol	Without chloramphenicol	With chloramphenicol
1	120	47	6 (−87)	35	36 (+3)
	240	622	33 (−95)	57	67 (+18)
2	120	79	19 (−76)	—	—
	240	1790	23 (−99)	—	—
3	120	—	—	187	158 (−16)
	240	—	—	223	184 (−17)
4	240	2260	34 (−99)	—	—
5	240	6300	32 (−99)	—	—
6	240	—	—	770	920 (+19)

Table 2. *Effect of chloramphenicol on the incorporation of [^{14}C]adenine into the nucleic acids and the trichloroacetic acid-soluble fraction of the subcellular fractions derived from rat-liver slices and cell suspensions*

Incubations were carried out in 8ml. of Ca^{2+}-free Krebs–Ringer phosphate buffer for 240min. The concentrations of chloramphenicol and [^{14}C]adenine were as given in Table 1. The total nucleic acid fraction (RNA+DNA) was isolated by method II (see the text).

Tissue preparation	Cell fraction	Specific activity of total nucleic acid fraction (RNA+DNA) (counts/min./mg.) Without chloramphenicol	With chloramphenicol	Change in the specific activity of total nucleic acids with chloramphenicol (%)	Total radioactivity in trichloroacetic acid-soluble fraction (counts/min.) Without chloramphenicol	With chloramphenicol	Change in the total radioactivity of trichloroacetic acid-soluble fraction with chloramphenicol (%)
Cell suspensions	Nuclear fraction	1450	60	−96	1270	808	−36
	Mitochondria	1220	32	−97	1320	876	−33
	Microsomes	0	0	—	142	87	−39
	Supernatant	517	70	−86	534	864	+62
Perfused liver slices	Nuclear fraction	410	312	−24	2050	1860	−9
	Mitochondria	171	148	−13	3110	3350	+8
	Microsomes	50	44	−12	349	309	−11
	Supernatant	344	294	−14	759	744	−2

necessary. All the radioactivities for ^{32}P-containing samples were corrected for decay to an initial specific activity of 1mc/mg. of P. The radioactivity in the 0hr. samples was 0 in every experiment when labelled adenine or uracil was used as the precursor. In experiments with [^{32}P]phosphate, correction was made for the small amount of radioactivity present in the 0hr. samples.

RESULTS

It has been reported (Jacob & Bhargava, 1965) that rat-liver cells in suspension can incorporate labelled bases into their RNA. Both the purine bases of RNA were shown to be labelled when [^{14}C]adenine was used as the precursor; both the pyrimidine bases of RNA (but not the thymine of DNA) were labelled when [^{14}C]orotic acid (or uracil, as was shown in subsequent experiments) was used as the precursor.

Chloramphenicol was found to inhibit almost completely the incorporation of [^{14}C]adenine or [^{14}C]uracil into the RNA of liver-cell suspensions whereas it had no significant inhibitory effect on the incorporation in the tissue slices (Tables 1 and 2). This was also shown by experiments determining the extent of the labelling of the total nucleic acids of the various cell fractions derived from the cell suspension and the tissue slices that had been incubated with the precursor (Tables 3 and 4); chloramphenicol inhibited the incorporation of the labelled bases into RNA of the various cell fractions derived from the cell suspensions by 80–100%, whereas with the tissue slices less than 25% inhibition of the incorporation of the precursors into RNA of any of the cell fractions was obtained.

It seemed possible that the lack of inhibition by chloramphenicol of RNA synthesis from labelled adenine and uracil in the tissue slices was due to a permeability barrier that did not exist in the cell suspensions. This was shown to be the case by experiments in which the effect of chloramphenicol on the incorporation of [^{14}C]adenine or [^{14}C]uracil into RNA of the homogenate derived from perfused liver slices was studied (Table 5); chloramphenicol almost completely inhibited the incorporation of either precursor into RNA of the homogenate.

Table 3. *Effect of chloramphenicol on the incorporation of [^{14}C]uracil into the RNA of rat-liver slices and cell suspensions*

Incubations were carried out in 4ml. of Ca^{2+}-free Krebs–Ringer phosphate buffer for 240min. The concentrations of chloramphenicol and [^{14}C]uracil were 50μg./ml. and 0·045μmole (0·46μc)/ml. respectively. The total nucleic acid fraction (RNA+DNA), from which RNA was obtained in the hydrolysed form, was isolated by method II (see the text). Values in parentheses give the percentage changes in the specific activity of RNA with chroramphenicol.

Expt. no.	Tissue preparation	Specific activity of RNA (counts/min./mg.) Without chloramphenicol	With chloramphenicol
1	Cell suspension	4860	0 (−100%)
2	Cell suspension	5700	25 (−99%)
3	Cell suspension	2300	20 (−99%)
4	Perfused liver slices	320	240 (−25%)

Table 4. *Effect of chloramphenicol on the incorporation of [^{14}C]uracil into the nucleic acids and the trichloroacetic acid-soluble fraction of the subcellular fractions derived from rat-liver slices and cell suspensions*

Incubations were carried out in 8ml. of Ca^{2+}-free Krebs–Ringer phosphate buffer for 240min. The concentration of chloramphenicol and [^{14}C]uracil were as given in Table 3. The total nucleic acid fraction (RNA+DNA) was isolated by method II (see the text).

Tissue preparation	Cell fraction	Specific activity of total nucleic acid fraction (RNA+DNA) (counts/min./mg.) Without chloramphenicol	With chloramphenicol	Change in the specific activity of total nucleic acids with chloramphenicol (%)	Total radioactivity in trichloroacetic acid-soluble fraction (counts/min.) Without chloramphenicol	With chloramphenicol	Change in the total radioactivity of trichloroacetic acid-soluble fraction with chloramphenicol (%)
Cell suspensions	Nuclear fraction	564	48	−92	570	522	−8
	Mitochondria	371	86	−77	1380	1150	−17
	Microsomes	105	0	−100	208	140	−30
	Supernatant	443	70	−84	543	978	+80
Perfused liver slices	Nuclear fraction	116	114	−1	579	540	−7
	Mitochondria	54	55	+2	863	842	−2
	Microsomes	53	50	−6	372	364	−2
	Supernatant	0	0	−6	84	88	+5

Table 5. *Effect of chloramphenicol on the incorporation of [^{14}C]adenine and [^{14}C]uracil into the RNA of homogenates derived from the perfused liver*

A 4ml. portion of the homogenate, prepared in Ca^{2+}-free Krebs–Ringer phosphate buffer as described in the text, was used for each time point. Incubations were carried out for 240min. [^{14}C]Adenine and [^{14}C]uracil concentrations were 0·45μc (0·49μmole)/ml. and 0·45μc (0·045μmole)/ml. respectively. The total nucleic acid fraction (RNA+DNA), from which RNA was obtained in the hydrolysed form, was isolated by method II (see the text). Values in parentheses give the percentage changes in the specific activity of RNA with chloramphenicol.

Precursor	Specific activity of RNA (counts/min./mg.) Without chloramphenicol	With chloramphenicol
[^{14}C]Adenine	2330	94 (−96)
[^{14}C]Uracil	161	29 (−82)

The inhibition of the incorporation of adenine and uracil into RNA by chloramphenicol in the cell suspensions could result from inhibition of transport of the bases from the extracellular medium to inside the cell, the transport of phosphate not being inhibited, inhibition of the conversion of the base into the corresponding nucleoside monophosphate, or inhibition of the polymerization of the nucleoside phosphates to RNA.

The first-mentioned possibility was not supported by experiments in which the transport of labelled adenine or uracil was studied (Tables 2, 4 and 6). As expected, chloramphenicol had no significant inhibitory effect (if at all, it had a stimulatory effect) on the uptake of [^{14}C]adenine or [^{14}C]uracil in the acid-soluble fraction(s) of either the tissue slices, or the various subcellular fractions derived from the tissue slices that had been incubated with the precursor. In the cell suspensions, the antibiotic did have a small but consistent inhibitory effect on such uptake of the precursor (except in the supernatant fraction in which the uptake was stimulated by 60–80% with both the precursors); the inhibition, however, was too low to account for the almost complete inhibition of the incorporation of the precursor into RNA.

In previous experiments it was shown that hepatic cells in suspension, like the tissue slices, could incorporate [^{32}P]phosphate into their RNA; the radioactivity contained in the 'hydrolysed RNA fraction' obtained by the method used in this study, after incubation of the cells with [^{32}P]phosphate, was shown to be accounted for almost entirely by the radioactivity of the individual constituent nucleotides separated electrophoretically (Jacob & Bhargava, 1965). In the present experiments, chloramphenicol was found not to have any inhibitory effect on the incorporation of [^{32}P]phosphate into RNA in the cell suspensions, the tissue slices or the homogenates derived from the tissue slices; on the contrary, the incorporation was stimulated by chloramphenicol in all the three tissue preparations in every experiment, often by over 100% (Table 7). In these experiments, the uptake of the precursor was measured by estimation of radioactivity in the alkaline hydrolysate (from which DNA had been removed by precipitation) of the total nucleic acid

Table 6. *Effect of chloramphenicol on the extent of radioactivity obtained in the trichloroacetic acid-soluble fraction derived from rat-liver slices and cell suspensions incubated with* [^{14}C]*adenine*

Incubations were carried out in 4ml. of Ca^{2+}-free Krebs–Ringer phosphate buffer for 240min. The concentrations of chloramphenicol and [^{14}C]adenine were the same as given in Table 1. Values in parentheses give the percentage changes in the total radioactivity of the trichloroacetic acid-soluble fraction with chloramphenicol.

	Total radioactivity of the trichloroacetic acid-soluble fraction (counts/min.)			
	Cell suspensions		Perfused liver slices	
Expt. no.	Without chloramphenicol	With chloramphenicol	Without chloramphenicol	With chloramphenicol
1*	109	51 (−53)	—	—
2	685	535 (−22)	—	—
3	—	—	1650	2600 (+57)
4	487	210 (−57)	6480	6700 (+3)

* Incubations were carried out for 120min.

Table 7. *Effect of chloramphenicol on the incorporation of* [^{32}P]*phosphate into the RNA of rat-liver slices, homogenates and cell suspensions*

Incubations were carried out in 3ml. of Krebs–Ringer bicarbonate buffer. The concentrations of chloramphenicol and [^{32}P]phosphate (including the phosphate of the buffer) were 50μg./ml. and 69·8μg. of P (33·3μc)/ml. respectively. The total nucleic acid fraction (RNA + DNA), from which RNA was obtained in the hydrolysed form, was isolated by method I (see the text). Values in parentheses give the percentage changes in the specific activity of RNA with chloramphenicol.

		Specific activity of RNA (counts/min./mg.)					
		Cell suspensions		Perfused liver slices		Homogenate from perfused liver	
Expt. no.	Period of incubation (min.)	Without chloramphenicol	With chloramphenicol	Without chloramphenicol	With chloramphenicol	Without chloramphenicol	With chloramphenicol
1	60	1520	3420 (+125)	3490	—	7950	12300 (+55)
	120	2920	6250 (+114)	7380	10300 (+40)	8000	19900 (+150)
	180	3690	8380 (+127)	9240	20800 (+125)	9310	37100 (+299)
2	60	—	—	1040	1950 (+87)	5290	7650 (+45)
	120	—	—	1110	2630 (+137)	6820	—
	180	—	—	3360	4340 (+29)	8790	11500 (+31)
3	60	1430	2550 (+78)	—	—	3750	4560 (+22)
	120	—	3700	—	—	7900	8650 (+10)
	180	3140	4800 (+53)	—	—	9760	11600 (+19)
4	90	970	2030 (+109)	2960	5970 (+102)	—	—
	180	1460	2870 (+97)	4700	12200 (+160)	—	—

fraction obtained by extraction with hot sodium chloride solution of the lipid-free trichloroacetic acid precipitate of the tissue preparation; that the stimulation of the incorporation of [^{32}P]phosphate by chloramphenicol represented a genuine stimulation of RNA synthesis was borne out by the finding that all the radioactivity in the 'hydrolysed RNA fraction' was accounted for (as in the absence of chloramphenicol) by the radioactivity in the constituent nucleotides separated electrophoretically (Table 8). These experiments clearly show that chloramphenicol does not inhibit the polymerization of the nucleoside phosphate to RNA.

The present investigation therefore strongly suggests that in the cell suspensions, in the presence of chloramphenicol, labelled adenine and uracil are not converted into the corresponding nucleoside monophosphates.

Table 8. *Distribution of radioactivity between the different nucleotides obtained by the hydrolysis of RNA derived from rat-liver slices and cell suspensions incubated with [^{32}P]phosphate in the presence of chloramphenicol*

The incubations were carried out for 3hr. in 3ml. of Krebs–Ringer bicarbonate buffer. The concentrations of chloramphenicol and [^{32}P]phosphate were as in Table 7. The total nucleic acid fraction (RNA+DNA) was isolated by method I and hydrolysed with alkali, and the resulting nucleotides were separated by electrophoresis, as described in the text.

	Specific activity of RNA (counts/min./mg.)		Specific activity of the nucleotides (counts/min./mg.)			
Type of preparation	Observed	Calc. from specific activities of the nucleotides*	Guanylic acid	Adenylic acid	Cytidylic acid	Uridylic acid
Perfused slices	5080	4700	4680	4850	4690	3160
Cell suspensions	2130	2050	1880	2290	2280	1220

* Based on the base ratio for RNA of liver reported by Fleck & Munro (1962).

DISCUSSION

Though no mammalian tissue has been previously reported to be permeable to chloramphenicol, this antibiotic has been shown to inhibit incorporation of certain precursors into RNA of two ascites-tumour cell suspensions (LePage, 1953) and of calf-thymus nuclei suspensions (Breitman & Webster, 1958). However, in both these investigations, the inhibitory effect was observed only at a very high chloramphenicol concentration (2000–2500 μg./ml.); in the former investigation, even at a concentration of 2500μg./ml., chloramphenicol caused only 40% inhibition of incorporation of labelled glycine into RNA adenine. The present investigation appears to be the first in which a low concentration (50 μg./ml.) of chloramphenicol has been shown to inhibit some step in the RNA-synthesizing mechanism (starting from the free base) in a mammalian system. This investigation also shows that cells that in the organized structure are impermeable to chloramphenicol may become permeable to it on dispersion of the tissue.

The present investigation further indicates that chloramphenicol exercises its inhibitory effect on RNA synthesis from adenine or uracil by preventing (directly or indirectly) the conversion of the base into the corresponding nucleoside or nucleotide. This view also finds support in the following obervations made by other workers:

(i) When net increase in the amount of RNA was taken as a criterion of RNA synthesis, RNA was found to continue to be synthesized (often at enhanced rates) in the presence of chloramphenicol (Gale & Folkes, 1953; LePage, 1953; Wissemann, Smadel, Hahn & Hopps, 1954; Pardee & Prestidge, 1956; Hahn, Schaechter, Ceglowski, Hopps & Caik, 1957; Horiuchi, Sunakawa & Mizuno, 1958; Harrington, 1958; Bernlohr & Webster, 1958; Gros & Gros, 1958; Aronson & Spiegelman, 1961; Yee, Pan & Gezon, 1962; Hahn & Wolfe, 1962).

(ii) Chloramphenicol does not inhibit the incorporation of [^{32}P]phosphate in *Bacillus megaterium*, *Escherichia coli*, calf-thymus nuclei, *Azotobacter vinelandii* and *Bacillus cereus* (Pardee, Paigen & Prestidge, 1957; Neidhart & Gros, 1957; Breitman & Webster, 1958; Chakravorty, 1960; Mandel & Altman, 1961), except, perhaps, at very high (over 2000 μg./ml.) concentrations.

(iii) Chloramphenicol, at concentrations up to 1000 μg./ml., does not significantly inhibit the incorporation of glycine into RNA in either ascites-tumour cells or *Azotobacter agilis* (LePage, 1953; Bernlohr & Webster, 1958). (The conversion of glycine into the purines of RNA does not involve the free base.)

(iv) Chloramphenicol has been shown to inhibit the incorporation of adenine and uracil into RNA in *B. cereus* (Mandel & Altman, 1961) and *Vibrio cholerae* (Kapoor *et al.* 1963).

This investigation does not throw any light on the mechanism by which chloramphenicol inhibits the conversion of the free base into the corresponding nucleoside monophosphate. The effect may be direct, such as on one (or more) of the enzymes involved in the above conversion (nucleoside phosphorylase, nucleoside kinase and nucleotide pyrophosphorylase), or indirect, such as on another process (like protein synthesis) that may be essential for RNA synthesis from free bases in the cell suspensions. At first sight, a direct effect on an enzyme system seems unlikely. Chloramphenicol is not known to be an inhibitor of enzymes at the concentration used; further, chloramphenicol does not seem to have any inhibitory effect on the activity of a purified preparation of nucleoside phosphorylase (Dr Richard Abrams, personal communication). On the other hand, chloramphenicol (50 μg./ml.)

has been shown to inhibit protein synthesis in liver cells in suspension by up to 50% (K. Bhargava & P. M. Bhargava, unpublished work).

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