SYNTHESIS AND PHYSICO-CHEMICAL PROPERTIES OF NEW ESTERS OF CHLORAMPHENICOL.
The esters were prepared by two methods:

METHOD I. - (Ch E₁ to Ch E₅)

A mixture of acid (0.195 mole) and thionyl chloride (40 ml) was refluxed for about 6 hours on a water bath. The excess of thionyl chloride was stripped off under reduced pressure. The crude acid chloride obtained was taken up in ethylene chloride (70 ml) and was used as such for condensation with chloramphenicol (0.087 mole) in the presence of pyridine (12 ml) in ethylene chloride solution at 70°C. The mixture was stirred at 70°C for 3 hours to get clear solution. It was cooled and washed with 0.1 N hydrochloric acid. It was further washed with 2% sodium bicarbonate solution followed by water. This was dried over anhydrous sodium sulphate. Ethylene chloride was stripped off under reduced pressure to get the final product. Recrystallisation with suitable solvents yields the desired compound.
METHOD II - (Ch E?)

The suspension of chloramphenicol (16.1 g, 0.05 mole) and N,N'-dicyclohexylcarbodiimide (10.0 g, 0.049 mole) in isobutyl methyl ketone (125 ml), was prepared and to this ampicillin (17.46 g, 0.05 mole) was added in parts slowly with stirring. The stirring was continued for 2 hours at temperature not exceeding 50°C. After this period, it was cooled and filtered. The filtrate was washed thrice with 2% sodium bicarbonate solution followed by washing with dilute hydrochloric acid and finally with water. This gave oily mass which was taken up into chloroform. The addition of petroleum ether (40-60°C) to the above solution, precipitated light yellow crystals. These were recrystallized from chloroform-petroleum mixture. The general method of synthesis of compounds is represented in Chart 1.

PHYSICO-CHEMICAL PROPERTIES

The new chloramphenicol esters were confirmed by elemental analysis, melting point, I.R., T.L.C., etc.

ELEMENTAL ANALYSIS:

Carbon, hydrogen and nitrogen were determined by Coleman analyzer.
CHART - i

I Method I
Stage I

\[
\text{Aromatic Acid} + \text{Thionyl Chloride} \rightarrow \text{Aromatic acid chloride}
\]

Stage II

\[
\text{Chloramphenicol} + \text{Pyridine} \rightarrow \text{Aroyl esters of chloramphenicol}
\]
Method: II

Chloramphenicol

\[ \text{O}_2\text{N} - \text{C} - \text{C} - \text{CH}_2 - \text{OH} + \text{HOC}\text{HCl} \]

\[ \text{HOOC} - \text{C} - \text{C} - \text{NH} - \text{CH} - \text{NH}_2 \]

Ampicillin

\[ \text{HNC} - \text{O} \]

\[ \text{DCC} \rightarrow -\text{H}_2\text{O} \]

Ampicillin ester of chloramphenicol.

Chart 1 - Synthesis of acid chloride and esters with chloramphenicol
DETERMINATION OF MELTING POINT:
The melting point of the compounds were determined by capillary method using Campbell melting point apparatus. The melting point of all new esters is given in respective monograph.

ULTRAVIOLET SPECTRUM:
About 25 mg of sample was weighed and was transferred in a dry 50 ml volumetric flask. It was dissolved and made up to mark with suitable solvent(s). It was further diluted with suitable solvent so as to obtain concentration 16 - 20 mcg/ml. The absorbancy of sample solution against blank was read from 230 - 400 nm on Beckman DU Spectrophotometer using 1 cm silica cell. The $E_{1\%}^{1cm}$ was calculated for each absorbancy and was plotted against wavelength. The $\lambda$ max was found out from the curve and was used for the esters for further study. The U.V. spectra of all seven compounds are given in plates 1 to 7.

I.R. SPECTRA:
8 to 10 mg of sample was mixed with 1.0 g of potassium bromide (previously dried at 120° C for 3 hours) in Agate mortar. A pellet of 150 to 160 mg was prepared from the potassium bromide mixture by pressing under pressure of 8 to 10 tons for 5 minutes.
PLATE - 7

UV Spectra of Compound Ch E₂
I.R. Spectra of the potassium bromide disc was taken from 2.5 to 25 micron of Perkin Elmer Grating Infrared Spectrophotometer, model No. 377, using scanning time 13 minutes, normal slit and auto time were kept constant.

The I.R. of all newly synthesized compounds are given in plates 8 to 14.

The comparative studies of I.R. of all the new compounds, with parent compounds have been tabulated in Table II.

Perusal of comparative group frequencies of the parent compound chloramphenicol and its seven esters makes the following points very clear:

1. -OH bending and -CO stretching at 1061 cm\(^{-1}\) is present only in chloramphenicol molecule, while it is found absent in all the compounds indicating that secondary OH alcoholic group has participated in ester formation.

2. With the exception of chloramphenicol, the parent compound, the peak in the range of 1750-1700 cm\(^{-1}\) has been observed in all the seven compounds indicating the formation of esters of chloramphenicol.
PLATE-11. I.R. SPECTRA OF COMPOUND Ch E₄

PLATE-12. I.R. SPECTRA OF COMPOUND Ch E₅

PLATE-13. I.R. SPECTRA OF COMPOUND Ch E₆
PLATE 14. I.R. SPECTRA OF COMPOUND Ch E7
DETERMINATION OF SOLUBILITY:

Weighed amounts of finely powdered compound were transferred to the dry test tubes. Measured volumes of solvents were added in small increments. After each addition, contents were stirred thoroughly with glass rod. Additions and stirrings were continued until almost clear solution was obtained. Total parts of solvents were calculated for 1 part of solute. The solubility of each compound in different solvents was carried out as above. Solubilities were expressed in different descriptive terms as follows:

The approximate solubility of substances are indicated by the following terms:

<table>
<thead>
<tr>
<th>Solubility in terms</th>
<th>Part of solvents required for 1 part of solute.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Very soluble</td>
<td>Less than 1</td>
</tr>
<tr>
<td>2) Freely soluble</td>
<td>From 1 to 10</td>
</tr>
<tr>
<td>3) Soluble</td>
<td>From 10 to 30</td>
</tr>
<tr>
<td>4) Sparingly soluble</td>
<td>From 30 to 100</td>
</tr>
<tr>
<td>5) Slightly soluble</td>
<td>From 100 to 1000</td>
</tr>
<tr>
<td>6) Very slightly soluble</td>
<td>From 1000 to 10000</td>
</tr>
<tr>
<td>7) Practically insoluble or</td>
<td></td>
</tr>
<tr>
<td>insoluble</td>
<td>More than 10000</td>
</tr>
</tbody>
</table>

The solubilities of all new esters in selected solvents are given in respective monographs.
SPECIFIC OPTICAL ROTATION:

About 0.5 g of sample was accurately weighed and was transferred in 25 ml volumetric flask containing about 20 ml of absolute alcohol. It was dissolved and the volume was made up to the mark with absolute alcohol. The angles of rotation of the solution and the blank by omitting the sample were measured at the wavelength of the sodium D-Line at about 20°C. The specific optical rotation was calculated as follows.

\[ \alpha^D_{20} = \frac{d \times 100}{L \times C} \]

Where

- \( \alpha \) = Observed angle of rotation
- \( L \) = Length of tube in decimeter.
- \( C \) = Concentration of solution in gm in 100 ml.

The specific optical rotation of all the compounds are given in the respective monographs.

pH

The pH of the 1% suspension of the compound in glass distilled water was determined by using Beckman pH Meter.
The pH of the compounds are included in respective monographs.

**BULK DENSITY:**

Weighed amount of the compound was transferred in clean dry cylinder. The cylinder was then tapped several times and the final volume occupied by the compound was noted. The bulk density was calculated by following formula:

$$\text{Bulk density} = \frac{\text{Weight of compound in g.}}{(g/ml) \text{ Volume occupied by compound in ml.}}$$

The bulk density of the compounds is included in the respective monographs.

**PARTICLE SIZE:**

500 mg of the dried compound was dispersed in oil in watch glass. From the dispersed suspension, few drops mounted on slide and the particle size of the compound was measured by the microscopic method. The particle size distributions of the compounds are represented in Graph 1.

**T.L.C.**

30 g of Kieselgel GF 254, Merck (Type 60) for thin layer chromatography was shaken vigorously with 60-65 ml water in a stoppered flask for 1 to 2 minutes to obtain uniform slurry.
Immediately, the slurry was poured into movable spreader. By means of spreader slurry was applied on glass plate (20x20 cm). The thickness of the layer obtained was 0.4 to 0.5 mm. The plates were left for 20 minutes at room temperature. Afterwards, the plates were transferred to a metal rack and the whole device was placed in drying oven (100-110°C) for 30 to 45 minutes. The plates were stored carefully to protect them from moisture and dust.

The solutions (4 mg/ml) of the new compounds and parent compounds were prepared in suitable solvents. 20 μl of compounds and 10 μl of parent compounds were spotted at 2.5 cm distance from the bottom of the plates by means of micropipette on T.L.C. plates. The spots were air dried. The top edge of chromato-plate was marked at 15 cm from the "STARTING LINE". Each chromato-plate was kept in the glass tank previously saturated with n-propyl alcohol and water (70:30). The chromatogram was developed by keeping the tank at room temperature until the mobile phase reached to 15 cm. Chromatoplate was removed and dried in hot air. Spots of the compounds were marked along with spots of parent compounds under U.V. exposure. In case of Ch E₇, the chromatoplate was sprayed with solution of 0.2% of ninhydrin in ethyl alcohol. The chromatoplate was dried in oven at 105°C for 10 minutes.
and spots of compounds were observed. The TLC plates of compounds (Ch E₁ to Ch E₇) are represented in plates 15 to 21.

**ASSAY:**

About 25 mg of the finely powdered and dried sample was accurately weighed and transferred in dry 50 ml volumetric flask. This was dissolved and made up to volume with suitable solvent(s). 1 ml of this solution was transferred in 25 ml volumetric flask and made up to mark with suitable solvent so as to set the concentration of the compound.

The absorbancy of sample against solvent blank was read at least at three different wave lengths in 1 cm silica cells on spectrophotometer. These wave lengths should show maximum absorbancy in curve. The \( E_{1\%}^{1\text{cm}} \) of the compound was calculated at three different wave lengths, \( E_{1\%}^{1\text{cm}} \) of the pure sample was considered to calculate the potency of corresponding sample.

The monographs of the compounds are tabulated in charts 2 to 8 and the summary of the same is tabulated in Table III.
A = Compound Ch E₁
B = Salicylic acid
C = Chloramphenicol

A = Compound Ch E₂
B = Acetyl salicylic acid
C = Chloramphenicol
PLATE-17

A = Compound Ch E^3
B = Paraamino salicylic acid
C = Chloramphenicol

PLATE-18

A = Compound Ch E^4
B = Paraamino benzoic acid
C = Chloramphenicol
T.L.C.

PLATE-19

A = Compound Ch E\textsubscript{5}
B = Para acetamido benzoic acid
C = Chloramphenicol

PLATE-20

A = Compound Ch E\textsubscript{6}
B = Nicotinic acid
C = Chloramphenicol
A = Compound Ch. B7
B = Ampicillin
C = Chloramphenicol
MONOGRAPH CHART - 2

COMPOUND Ch E₁

DESCRIPTION Pink coloured, fine crystalline hygroscopic tasteless powder.

SOLUBILITY It is slightly soluble in water, sparingly soluble in ethanol, methanol, chloroform and soluble in acetone.

pH 5.6

BULK DENSITY 0.5513 g/cc.

MELTING POINT 70–72°C

OPTICAL ROTATION \(\alpha\)D \(+14.96\°

ELEMENTAL ANALYSIS

<table>
<thead>
<tr>
<th>Element</th>
<th>Required (%)</th>
<th>Found (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>48.76</td>
<td>49.20</td>
</tr>
<tr>
<td>H</td>
<td>3.61</td>
<td>3.90</td>
</tr>
<tr>
<td>N</td>
<td>6.32</td>
<td>6.00</td>
</tr>
</tbody>
</table>

MOLECULAR FORMULA: \(\text{C}_18\text{H}_{16}\text{N}_2\text{O}_7\text{Cl}_2\) Molecular weight: 443

U.V. Spectrum Maxima \(E_{1\text{cm}}^{1\%}\)
235 nm 341.2
273 nm 197.0

I.R. Spectrum (KBr) Wave number (cm\(^{-1}\))
3400 - 3250
1725, 1515,
1690, 1350,
1610.

STRUCTURE

\[2-[(\alpha,\alpha',-\text{Dichloroacetyl})\text{amino}]3-\text{hydroxy}-3-(4'\text{-nitrophenyl})\text{ propyl salicylate}\]
MONOGRAPH CHART - 3

COMPOUND: Ch E

DESCRIPTION: Tasteless yellowish or yellowish brown coloured crystalline powder having hygroscopic property.

SOLUBILITY: It is slightly soluble in water, freely soluble in ethanol, methanol, acetone and chloroform.

pH: 4.0

BULK DENSITY: 0.5599 g/cc.

MELTING POINT: 68-71° C

OPTICAL ROTATION: \( [\alpha]_D^{20} + 9.94^\circ \)

ELEMENTAL ANALYSIS:

<table>
<thead>
<tr>
<th>Required (%)</th>
<th>Found (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N: 4.87</td>
<td>4.80</td>
</tr>
</tbody>
</table>

MOLECULAR FORMULA: \( C_{20}H_{18}N_2O_8Cl \cdot 5H_2O \)

Molecular weight: 575

U.V. Spectrum:
- Maxima: \( E_\lambda \) cm
  - 230 nm: 280.67
  - 270 nm: 140.99

I.R. Spectrum (KBr) Wave number (cm\(^{-1}\))
- 3400 - 3300
- 1740, 1520,
- 1690, 1350,
- 1610,

STRUCTURE:

\[
\begin{align*}
\text{O}_2N & \quad \text{H} & \quad \text{NHCOCH}_2 & \quad \text{O} \\
\text{C} & \quad \text{C} & \quad \text{CH}_2 & \quad \text{O} & \quad \text{C} & \quad \text{C} & \quad \text{CH}_3
\end{align*}
\]

2-[(d,l, -Dichloroacetyl)amino]-3-hydroxy-3-(4'-nitrophenyl) propyl-2'-acetylsalicylate.
**MONOGRAPH CHART - 4**

**COMPOUND**  
Ch E₃

**DESCRIPTION**  
Tasteless yellowish or buff brown coloured fine crystalline powder.

**SOLUBILITY**  
It is soluble in water, slightly soluble in ethanol, chloroform, sparingly soluble in methanol and acetone.

**pH**  
7.8

**BULK DENSITY**  
0.5535 g./cc.

**MELTING POINT**  
204 - 206°C

**ELEMENTAL ANALYSIS**

<table>
<thead>
<tr>
<th>Required (%)</th>
<th>Found (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C : 47.13</td>
<td>46.50</td>
</tr>
<tr>
<td>H : 3.71</td>
<td>4.00</td>
</tr>
<tr>
<td>N : 9.16</td>
<td>9.30</td>
</tr>
</tbody>
</table>

**MOLECULAR FORMULA**  
C₁₈H₁₇N₃O₇Cl₂  
Molecular weight : 458

**U.V. Spectrum**

<table>
<thead>
<tr>
<th>Maxima</th>
<th>E₁ cm</th>
<th>1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>305 nm</td>
<td>353.13</td>
<td>1%</td>
</tr>
</tbody>
</table>

**I.R. Spectrum (KBr)**

<table>
<thead>
<tr>
<th>Wave number (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3460-3230</td>
</tr>
<tr>
<td>1705, 1520, 1640, 1350, 1610</td>
</tr>
</tbody>
</table>

**STRUCTURE**

2-[(α, α, -Dichloroacetyl)amino]-3-hydroxy-3-(4'-nitrophenyl) propyl-4'-aminosalicylate.
MONOGRAPH CHART - 5

COMPOUND

Ch E₄

DESCRIPTION

Tasteless yelbwish to yellowish brown, fine crystalline powder.

SOLUBILITY

It is insoluble in water, chloroform, sparingly soluble in ethanol, soluble in methanol and freely soluble in acetone.

PH

4.7

BULK DENSITY

0.6592 g/cc.

MELTING POINT

78-81° C

ELEMENTAL ANALYSIS

<table>
<thead>
<tr>
<th>Required (%)</th>
<th>Found (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C : 48.87</td>
<td>50.09</td>
</tr>
<tr>
<td>H : 3.84</td>
<td>4.1</td>
</tr>
<tr>
<td>N : 9.46</td>
<td>9.90</td>
</tr>
</tbody>
</table>

MOLECULAR FORMULA

C₁₈H₁₇N₃O₆Cl₂

Molecular weight : 442

U.V. Spectrum

Maxima 1% E₁ cm⁻¹
235 nm  222.22
310 nm  463.7

I.R. Spectrum (KBr)

Wave number (cm⁻¹)
3440-3200
1690, 1517,
1630, 1350,
1605,

STRUCTURE

2-[α,α,α-Dichloroacetyl) amino]-3-hydroxy-3-(4'-nitrophenyl) propyl-4"-aminobenzoate.
MONOGRAPH CHART - 6

COMPOUND: Ch E5

DESCRIPTION: Almost tasteless, slight bitter after taste, grey buff coloured fine crystalline powder.

SOLUBILITY: It is insoluble in water, ethanol, methanol, chloroform and soluble in acetone.

pH: 4.5

BULK DENSITY: 0.3879 g/cc.

MELTING POINT: 205-208° C

ELEMENTAL ANALYSIS:

<table>
<thead>
<tr>
<th>Required (%)</th>
<th>Found (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C : 49.60</td>
<td>49.10</td>
</tr>
<tr>
<td>H : 3.93</td>
<td>4.40</td>
</tr>
<tr>
<td>N : 8.68</td>
<td>8.50</td>
</tr>
</tbody>
</table>

MOLECULAR FORMULA: C20H19N3O7Cl2

Molecular weight: 484

U.V. Spectrum:

Maxima E1cm

275 nm 669.4

I.R. Spectrum (KBr):

Wave number (cm⁻¹)

3400-3340

1725, 1530,

1680, 1350,

1605.

STRUCTURE:

2 - [(α,α'-Dichloroacetyl)amino]-3-hydroxy-3-(4'-nitrophenyl) propyl-4''-acetamidobenzoate.
MONOGRAPH CHART - 7

**COMPOUND**

Ch E₆

**DESCRIPTION**

It is pinkish, free flowing, crystalline powder having bitter taste.

**SOLUBILITY**

It is slightly soluble in water, chloroform, sparingly soluble in methanol, soluble in ethanol and freely soluble in acetone.

**pH**

4.8

**BULK DENSITY**

0.4395 g/cc.

**MELTING POINT**

153-155°C

**OPTICAL ROTATION**

[^D]_D^20° + 39.13

**ELEMENTAL ANALYSIS**

<table>
<thead>
<tr>
<th>Required (%)</th>
<th>Found (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C : 47.66</td>
<td>47.70</td>
</tr>
<tr>
<td>H : 3.50</td>
<td>3.46</td>
</tr>
<tr>
<td>N : 9.81</td>
<td>9.50</td>
</tr>
</tbody>
</table>

**MOLECULAR FORMULA**

C₁₇H₁₅N₃O₆Cl₂

Molecular weight: 428

**U.V. Spectrum**

Maxima E₁% cm⁻¹

270 nm 291.94

**I.R. Spectrum (KBr)**

Wave number (cm⁻¹)

3390,
1730, 1520,
1662, 1350,
1590,

**STRUCTURE**

2-[(L,L, -Dichloroacetyl)-amino]-3-hydroxy-3-(4'-nitrophenyl) propyl nicotinate.
**MONOGRAPH CHART - 8**

**COMPOUND**
Ch E7

**DESCRIPTION**
Tasteless, light yellow, crystalline, free flowing powder.

**SOLUBILITY**
It is slightly soluble in water, sparingly soluble in ethanol, methanol, soluble in acetone and freely soluble in chloroform.

**pH**
6.8

**BULK DENSITY**
0.5921 g / cc.

**MELTING POINT**
78-81° C

**OPTICAL ROTATION**
$\left( \alpha \right)_D^{20^\circ} + 14.9$

**ELEMENTAL ANALYSIS**

<table>
<thead>
<tr>
<th>Required (%)</th>
<th>Found (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C : 49.54</td>
<td>50.72</td>
</tr>
<tr>
<td>H : 4.43</td>
<td>4.61</td>
</tr>
<tr>
<td>N : 10.70</td>
<td>10.91</td>
</tr>
</tbody>
</table>

**MOLECULAR FORMULA**
C$_{27}$H$_{29}$N$_5$O$_8$SCl$_2$

Molecular weight : 653.92

**U.V. Spectrum**
Maxima

$\varepsilon_{1cm}$

275 nm 128.96

**I.R. Spectrum (KBr)**
Wave number $(\text{cm}^{-1})$

3340 - 3290
1745, 1510,
1660, 1340.

**STRUCTURE**

2-[(L,L, -Dichloroacetyl) amino]-3-hydroxy-3 (4'-nitrophenyl) propyl-6''-(2'' -amino-2'' -phenyl acetamido)-3'', 3'' -dimethyl -7''-oxo-4''-thia -1''-azabicyclo (3,2,0) heptane-2''-carboxylate.
Whenever a drug is made, some assurance is required to the effect that it will remain substantially unchanged for a reasonably long period of time. When stored at different conditions, it will naturally affect the stability of the drug.

It is essential that the drug must be pure before any stability tests are initiated. An impurity may catalyze the breakdown of a drug or it might be unstable itself, altering the physical appearance of a perfectly stable drug substance.

Instability may appear by a number of different ways like hydrolysis, oxidation, colour change due to physico-chemical reactions, chemical decomposition under the different storage conditions like temperature, light, humidity, etc.
Stability tests are designed to investigate the effect of temperature and moisture upon the stability of the pure drug. They also determine the proper handling and storage techniques. Compounds are monitored regularly for physical changes, water content and chemical degradation.

ACCELERATED STABILITY TESTING OF DRUGS

There appears to be a wide gap between two methods of approach to stability testing. One approach which is symptomatic of the early stages of the subject, employs a detailed technical approach to simple systems. The other, which of necessity has to be frequently used in an ad-hoc storage of samples under various conditions with decision based on subjective impressions.

All these applications need to use the technique of speeding up the rate at which something happens, that is probing the weakness and relating this instability pattern under higher stress and longer time. The technique has at least two defects:

1. Under the chosen high stress something may happen which never occurs under lower stress; and

2. The relationship between the high stress/short time and the low stress/long time must be established.
The effect of humidity on the stability of the drug can be studied by exposing the drug in various cabinets of varying range of humidities. The various ranges of humidities can be achieved by employing saturated solution of required salts.

Effect of temperature on the stability of the drug can be studied by exposing the product at various temperature and examining at decided intervals for deterioration involving various types of the changes.

EXPERIMENTAL:

INFLUENCE OF VARYING HUMIDITIES AND TEMPERATURE ON DRUGS IN POWDER FORM:

In order to study the influence of humidity and temperature on compounds, they were exposed to varying sets of humidities and temperatures. Both physical change and change in the potency of the powder form were studied.

PREPARATION OF DESSICATORS OF VARYING HUMIDITIES

Saturated solution of calcium chloride of required concentration was kept in dessicators to get the following
range of humidities.

<table>
<thead>
<tr>
<th>% Humidity</th>
<th>% of Calcium Chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>14.95</td>
</tr>
<tr>
<td>80</td>
<td>22.25</td>
</tr>
<tr>
<td>70</td>
<td>27.40</td>
</tr>
<tr>
<td>50</td>
<td>35.64</td>
</tr>
<tr>
<td>30</td>
<td>44.36</td>
</tr>
<tr>
<td>20</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Blanks were kept in the dessicators containing calcium chloride to get almost moisture-free environment inside the dessicators.

**STORAGE OF COMPOUNDS UNDER DIFFERENT HUMIDITIES:**

All compounds were dried in a dessicator containing calcium chloride under vacuum (23 mm Hg) for a period of 3 hours to remove moisture. Required amount of compounds were spread evenly on plastic disc (5 cm diameter). The discs so prepared were placed on the perforated plates of the dessicators offering different humidities.

The compounds exposed to varying humidities such as 90%, 80%, 70%, 50%, 30% and 20% were withdrawn after
3, 6, 15, 30, 45, 60, 75 and 90 days. All the samples were dried under vacuum in a dessicator containing calcium chloride and the analysis was carried out as described earlier.

**STORAGE AT THE VARIOUS TEMPERATURES:**

Sets of compounds filled in vials were kept at 45°C, 37°C and 5°C ± 1°C. The samples were withdrawn at an interval of 15, 30, 45, 60, 75 and 90 days and analysis was carried out.

**RESULTS:**

**INFLUENCE OF HUMIDITY ON THE STABILITY OF ESTERS:**

Results of the influence of humidity range 20% RH to 90% as shown in Tables IV to X. The results of all the compounds are expressed as (I) % retention, and confirmed further by (II) optical rotation measurement and (III) Max $E_{1\text{ cm}}^{1%}$ initially and after 3, 6, 15, 30, 45, 60, 75 and 90 days. The comparison of I.R. initially and taken after 90 days as shown in plates 22 to 28 confirms the findings.

The comparison of the percentage retention of all the esters was carried out at the end of 90 days at 90% humidity. The results are expressed as under.
PLATE-22. I.R. SPECTRA OF COMPOUND Ch E₁

PLATE-23. I.R. SPECTRA OF COMPOUND Ch E₂

PLATE-24. I.R. SPECTRA OF COMPOUND Ch E₃
PLATE-25. I.R. SPECTRA OF COMPOUND Ch E₄

PLATE-26. I.R. SPECTRA OF COMPOUND Ch E₅

PLATE-27. I.R. SPECTRA OF COMPOUND Ch E₆
COMPARISON OF PERCENTAGE RETENTION OF ESTERS AT THE END OF 90 DAYS AT 90% HUMIDITY:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ch E₁</th>
<th>Ch E₂</th>
<th>Ch E₃</th>
<th>Ch E₄</th>
<th>Ch E₅</th>
<th>Ch E₆</th>
<th>Ch E₇</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Retention</td>
<td>97.95</td>
<td>85.02</td>
<td>99.18</td>
<td>98.87</td>
<td>93.98</td>
<td>101.12</td>
<td>97.33</td>
</tr>
<tr>
<td>Grade</td>
<td>4</td>
<td>7</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

The results indicate that out of all the seven compounds, compound Ch E₆ is found to be maximum stable. The sequence of stability of other compounds is expressed in descending order as Ch E₃, Ch E₄, Ch E₁, Ch E₇, Ch E₅ and Ch E₂.

At the maximum RH of 90% and at the end of 3 months period the most unstable compound amongst these seven compounds - Ch E₂ - lost only 15% of its activity.

The probable destruction of compound Ch E₂ may be explained on the following pattern of degradation as noticed on TLC plate 29.
A = Compound Ch E_2
B = Acetylsalicylic acid
C = Salicylic acid
D = Chloramphenicol
Compound Ch E₂ when kept at humidity 90% for 90 days showed an additional spot corresponding to that of salicylic acid on a TLC plate. This suggests that due to high humidity, the product Ch E₂ undergoes deacetylation and hydrolysis which are induced by water within 45 days. From this time onwards the spot for salicylic acid starts showing up in TLC and is at its peak in 90 days. The TLC examination showed only two spots - one due to compound and other due to salicylic acid - indicating that deacetylation and hydrolysis start simultaneously.

It may be concluded that all the compounds are reasonably stable under the influence of humidity.

After 90 days, equal quantity of all the compounds were withdrawn and were subjected to the determination of moisture content by K.F. method. The moisture retention i.e. hygroscopicities shown by these compounds showed the same order as shown by the percentage retention of antibiotic at 90% humidity at the end of 90 days indicating that the compounds having highest hygroscopicity was more liable to destruction as compared to those which were less hygroscopic.
MOISTURE CONTENT OF THE ESTERS AT THE END OF NINTY DAYS AT 90% RH.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ch E₁</th>
<th>Ch E₂</th>
<th>Ch E₃</th>
<th>Ch E₄</th>
<th>Ch E₅</th>
<th>Ch E₆</th>
<th>Ch E₇</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content (in %)</td>
<td>1.74</td>
<td>2.85</td>
<td>0.18</td>
<td>1.92</td>
<td>0.2</td>
<td>0.28</td>
<td>2.12</td>
</tr>
<tr>
<td>Grade</td>
<td>4</td>
<td>1</td>
<td>7</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

INFLUENCE OF TEMPERATURE ON THE STABILITY OF ESTERS:
The exposures of compounds at 5°C, 37°C, and 45°C at decided time intervals for the period of ninty days as shown in Tables XI to XVII do not show significant destruction of esters but for accelerated studies, % retention at 45°C at the end of ninty days is taken into consideration. The comparative results of the same are as under:

COMPARISON OF % RETENTION OF ESTERS AT THE END OF 90 DAYS AT 45°C TEMPERATURE.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ch E₁</th>
<th>Ch E₂</th>
<th>Ch E₃</th>
<th>Ch E₄</th>
<th>Ch E₅</th>
<th>Ch E₆</th>
<th>Ch E₇</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Retention</td>
<td>100.00</td>
<td>98.16</td>
<td>100.23</td>
<td>98.58</td>
<td>98.21</td>
<td>99.93</td>
<td>96.03</td>
</tr>
<tr>
<td>Grade</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Temperature</td>
<td>Content in %</td>
<td>TIME IN DAYS</td>
<td>Maxima</td>
<td>E1%cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>-------------</td>
<td>--------------</td>
<td>--------</td>
<td>--------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45°C</td>
<td>98.86</td>
<td>100.02</td>
<td>305,349,12</td>
<td>305,351.13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37°C</td>
<td>99.80</td>
<td>99.42</td>
<td>305,355,21</td>
<td>305,350.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5°C</td>
<td>99.61</td>
<td>97.53</td>
<td>305,351,78</td>
<td>305,344.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>98.88</td>
<td></td>
<td>305,354.21</td>
<td>305,349.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>98.68</td>
<td></td>
<td>305,354.21</td>
<td>305,354.21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Optical Rotation was not measurable.
TABLE - XIV

STUDIES ON EFFECT OF VARYING TEMPERATURE ON COMPOUND Ch E₄ FOR THE TOTAL PERIOD OF 90 DAYS

<table>
<thead>
<tr>
<th>Temperature</th>
<th>I Content in %</th>
<th>III Maxima E₁cm</th>
<th>TIME IN DAYS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15  30  45  60  75  90</td>
<td>15  30  45  60</td>
<td></td>
</tr>
<tr>
<td>5°C</td>
<td>100.44 102.47 100.69 102.15 98.91 101.20</td>
<td>310,465.73 310,475.17 310,466.89 310,473.67</td>
<td></td>
</tr>
<tr>
<td>37°C</td>
<td>100.73 102.23 100.75 100.86 100.64 98.24</td>
<td>310,467.10 310,474.04 310,467.16 310,467.72</td>
<td></td>
</tr>
<tr>
<td>45°C</td>
<td>101.19 101.50 100.50 99.85 98.37 98.58</td>
<td>310,469.20 310,470.90 310,466.36 310,462.98</td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>100</td>
<td></td>
<td>310,463.70</td>
</tr>
</tbody>
</table>

II Optical rotation was not measurable
<table>
<thead>
<tr>
<th>Temperature</th>
<th>I Content in %</th>
<th>TIME IN DAYS</th>
<th>III</th>
<th>Maxima</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 30 45 60 75 90</td>
<td>15 30 45 60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5° C</td>
<td>103.49 99.22 95.78 94.44 98.11 99.58</td>
<td>275,672.71 275,664.18 275,661.23 275,668.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37° C</td>
<td>100.26 97.60 96.02 99.19 100.20 97.63</td>
<td>275,671.11 275,652.12 275,656.14 275,663.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45° C</td>
<td>104.41 99.09 99.33 97.89 97.74 98.21</td>
<td>275,608.95 275,663.22 275,604.99 275,655.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>100</td>
<td>275,662.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

II Optical rotation was not measurable.
The above data shows that compound Ch E₃ is showing maximum stability followed by compound Ch E₁, Ch E₆, Ch E₄, Ch E₅, Ch E₂ and Ch E₇ in the descending order. The compound Ch E₇ showed only 4% destruction at 45°C at the end of 90 days. This can be considered as negligible at proper storage conditions.

The comparison of I.R. spectra taken initially and after ninety days is given in plates 30 to 36 which confirms the earlier findings.

**STABILITY OF NEW ESTERS WITH SELECTED ADDITIVES COMMONLY EMPLOYED IN THE FORMULATIONS.**

Stability studies were designed with selected additives namely Starch, Lactose, Dicalcium Phosphate, Magnesium Stearate and Sodium CMC. 181.8 mg of each ester was combined with 2 g of selected additives. The mixtures were filled in sealed vials and were exposed at 45°C in an incubator for a period of 30 days. The content of compound was estimated initially and at the end of 30 days. The comparative results of stability studies are as under:
PLATE-30. I.R.SPECTRA OF COMPOUND Ch E₁

PLATE-31. I.R.SPECTRA OF COMPOUND Ch E₂

PLATE-32. I.R.SPECTRA OF COMPOUND Ch E₃
PLATE 33. I.R. SPECTRA OF COMPOUND Ch E₄

PLATE 34. I.R. SPECTRA OF COMPOUND Ch E₅

PLATE 35. I.R. SPECTRA OF COMPOUND Ch E₆
PLATE-36. I.R. SPECTRA OF COMPOUND CH 27
STABILITY STUDIES ON COMPOUNDS WITH SELECTED ADDITIVES AT 45°C INITIALLY AND AT THE END OF 30 DAYS

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time in days</th>
<th>Starch</th>
<th>Lactose</th>
<th>Dicalcium Phosphate</th>
<th>Magnesium Stearate</th>
<th>Sodium CMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch E₁</td>
<td>0</td>
<td>104.64</td>
<td>105.21</td>
<td>103.54</td>
<td>104.67</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>101.54</td>
<td>102.14</td>
<td>101.37</td>
<td>103.50</td>
<td>97.68</td>
</tr>
<tr>
<td>Ch E₂</td>
<td>0</td>
<td>102.76</td>
<td>103.75</td>
<td>102.34</td>
<td>102.10</td>
<td>103.25</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>99.85</td>
<td>101.21</td>
<td>100.08</td>
<td>101.85</td>
<td>101.70</td>
</tr>
<tr>
<td>Ch E₃</td>
<td>0</td>
<td>100.89</td>
<td>103.71</td>
<td>102.15</td>
<td>101.97</td>
<td>102.27</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>98.61</td>
<td>102.99</td>
<td>100.75</td>
<td>99.77</td>
<td>100.08</td>
</tr>
<tr>
<td>Ch E₄</td>
<td>0</td>
<td>104.12</td>
<td>104.40</td>
<td>104.09</td>
<td>103.35</td>
<td>98.45</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>103.10</td>
<td>102.39</td>
<td>103.80</td>
<td>102.34</td>
<td>98.02</td>
</tr>
<tr>
<td>Ch E₅</td>
<td>0</td>
<td>99.20</td>
<td>99.33</td>
<td>100.71</td>
<td>98.34</td>
<td>97.65</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>98.51</td>
<td>98.29</td>
<td>99.94</td>
<td>97.75</td>
<td>97.13</td>
</tr>
<tr>
<td>Ch E₆</td>
<td>0</td>
<td>100.38</td>
<td>103.52</td>
<td>103.02</td>
<td>104.70</td>
<td>98.90</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>99.85</td>
<td>101.75</td>
<td>102.50</td>
<td>104.20</td>
<td>97.52</td>
</tr>
<tr>
<td>Ch E₇</td>
<td>0</td>
<td>100.20</td>
<td>103.68</td>
<td>102.42</td>
<td>99.83</td>
<td>99.21</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>99.85</td>
<td>99.75</td>
<td>99.91</td>
<td>99.61</td>
<td>98.85</td>
</tr>
</tbody>
</table>
The percentage retention of each ester with selected diluents was calculated as under:

**COMPARISON OF PERCENTAGE RETENTION OF NEW ESTERS WITH ADDITIVES AT 45°C AT THE END OF 30 DAYS.**

<table>
<thead>
<tr>
<th>Additive</th>
<th>Ch E₁</th>
<th>Ch E₂</th>
<th>Ch E₃</th>
<th>Ch E₄</th>
<th>Ch E₅</th>
<th>Ch E₆</th>
<th>Ch E₇</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch</td>
<td>97.03</td>
<td>97.16</td>
<td>97.74</td>
<td>99.02</td>
<td>99.30</td>
<td>99.47</td>
<td>99.65</td>
</tr>
<tr>
<td>Lactose</td>
<td>97.68</td>
<td>97.55</td>
<td>99.30</td>
<td>98.07</td>
<td>98.95</td>
<td>98.29</td>
<td>96.20</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
<td>97.90</td>
<td>97.79</td>
<td>98.62</td>
<td>99.72</td>
<td>99.23</td>
<td>99.49</td>
<td>97.54</td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td>98.88</td>
<td>99.75</td>
<td>97.84</td>
<td>99.02</td>
<td>99.40</td>
<td>99.52</td>
<td>99.77</td>
</tr>
<tr>
<td>Sodium CMC</td>
<td>97.68</td>
<td>98.49</td>
<td>97.85</td>
<td>99.56</td>
<td>99.46</td>
<td>98.60</td>
<td>99.63</td>
</tr>
</tbody>
</table>

The above results show that percentage retention of the compound after 30 days exposure lies between 96 to 99. So, it can be concluded that all new esters are reasonably stable.

**INFLUENCE OF LIGHT ON NEW ESTERS.**

Stability studies on new esters were carried out by exposing them in fine state of division in 5 ml white
glass vials in the light cabinet for the period of 15 days at 45°C. The content of compound was estimated initially and at the end of fortnight. The light cabinet having six, two feet fluorescent tubes 20 watts each and six 60 watts clear coiled filament lamps giving about 35 times more light than the light falling in the laboratory and temperature maintained at 45°C was used. The results are as under:

INITIAL CONTENT AND THE CONTENT AFTER 15 DAYS ALONG-WITH PERCENTAGE RETENTION, UNDER INFLUENCE OF LIGHT AND TEMPERATURE AT 45°C.

<table>
<thead>
<tr>
<th></th>
<th>Ch E₁</th>
<th>Ch E₂</th>
<th>Ch E₃</th>
<th>Ch E₄</th>
<th>Ch E₅</th>
<th>Ch E₆</th>
<th>Ch E₇</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>101.30</td>
<td>100.21</td>
<td>103.72</td>
<td>102.55</td>
<td>102.10</td>
<td>99.85</td>
<td>99.45</td>
</tr>
<tr>
<td>After 15 days at 45°C</td>
<td>99.25</td>
<td>99.75</td>
<td>101.35</td>
<td>101.35</td>
<td>99.92</td>
<td>98.25</td>
<td>97.45</td>
</tr>
<tr>
<td>% Retain after 15 days at 45°C</td>
<td>97.97</td>
<td>99.54</td>
<td>97.71</td>
<td>98.82</td>
<td>97.86</td>
<td>98.39</td>
<td>97.98</td>
</tr>
</tbody>
</table>

The above results show that retention of the compound after 15 days exposure lies between 97 to 99. So, it can be concluded that all new esters are reasonably stable to the light.
BIOLOGICAL & PHARMACOLOGICAL PROPERTIES

ESTIMATION OF MINIMUM INHIBITORY CONCENTRATION & ANTIMICROBIAL ACTIVITY OF NEW ESTERS ON SELECTED MICROBES.
The anti-microbial activity of the compounds is influenced by several factors. While screening of such compounds, all possible factors should be taken into consideration. Composition of testing medium, hydrogen ion concentration, size of the inoculam, concentration of the compound etc. affect the activity of the compounds. In the present investigations by keeping all the factors constant, the antibacterial activity of the compounds was screened in acidic and alkaline medium.

MINIMUM INHIBITORY CONCENTRATION:

The minimum inhibitory concentrations of newly synthesised chloramphenicol esters were estimated by agar cup-plate method\(^{52}\) against selected Gram-positive and Gram-negative organisms and yeasts, and were compared with that of chloramphenicol and chloramphenicol palmitate.

METHOD:

Specially designed glass plates, 24 cm x 24 cm x 0.8 cm
were employed instead of petri dishes. 150 ml of the medium was inoculated with 24 hours old culture of each organism (except yeast cultures which were 48 hours old) and was poured in the plates, placed on the levelled surface. 25 cups were bored each of 6 mm diameter, which were filled with fresh solutions of chloramphenicol esters, along with chloramphenicol in DMF, in 0.05 ml quantities, DMF as such was also placed as blank (the range of the concentrations selected lies between 0.001 to 0.4 mcg/ml). The plates were incubated at 32°C for bacterial cultures and 22°C for the yeast cultures. The results were recorded after 18 hrs for bacterial cultures and after 48 hours for the yeast cultures. Inhibitory concentrations are presented in Table XVIII.

Compound Ch E₁ is found to be the most active against varieties of yeast viz. S.cerevisiae, S.carlsbergensis and C.albicans. It has been found to have similar activity against Gram-negative strains of E.coli and three Gram-positive strains of B.subtilis, S.aureus and M.flavus. It has little less activity against Gram-positive strains of B.pumilus and Gram-negative strains of K.pneumoniae and P.aeruginosa.

Compound Ch E₂ is found to be the most active against two strains of E.coli, B.subtilis, M.flavus and C.albicans. It has little lower activity against S.aureus, S.cerevisiae
and *S.carlsbergensis*. The lowest activity is found against *B.pumilus, K.pneumoniae* and *P.aeruginosa*.

Compound Ch $E_3$ is active against two strains of *E.coli, B.subtilis, M.flavus* and *S.cerevisiae*. It is less active against *B.pumilus, K.pneumoniae, P.aeruginosa* and *S.aureus* and minimum activity against *S.carlsbergensis*.

The activity of compound Ch $E^#$ is found of the same order against both the strains of *E.coli, B.subtilis, M.flavus, C.albicans, K.pneumoniae, S.aureus, S.cerevisiae, S.carlsbergensis*. It was little less active against *B.pumilus* and *P.aeruginosa*.

The compound Ch $E_5$ is found more active against all organisms selected with the exception of *P.aeruginosa* and *S.carlsbergensis* where activity was bit inferior.

The compound Ch $E_6$ showed maximum activity against *M.flavus, C.albicans* followed by *S.aureus, B.subtilis, E.coli, K.pneumoniae, B.pumilus, S.carlsbergensis* and *S.cerevisiae* in descending order.

Compound Ch $E_7$ was found highly active against two strains of *E.coli, B.subtilis, C.albicans* and *M.flavus*. Lesser
activity was observed against B. pumilus, K. pneumoniae, P. aeruginosa, S. aureus, S. cerevisiae, S. carlsbergensis.

As compared to the minimum inhibitory concentrations exhibited by chloramphenicol palmitate and chloramphenicol, the newly synthesized compounds showed nearly comparable inhibitory concentrations range. Comparison of new seven compounds showed little inferiority of compound Ch E6. All the new compounds were found effective against selected sets of organisms.

THE INVITRO ACTIVITY OF NEWLY SYNTHESIZED ESTERS AT ACIDIC AND ALKALINE pH AGAINST SELECTED ORGANISMS.

Chloramphenicol esters Ch E1 to Ch E7 synthesized in this lab were tested against Gram-positive, Gram-negative and Yeast at acidic pH (4.5) and in alkaline pH (8.0) to study their effectiveness at extreme conditions of pH and were compared with chloramphenicol palmitate and chloramphenicol under the same sets of conditions.

METHOD:

Agar cup-plate method was used to study the influence of acidic and alkaline pH of the esters against selected organisms.

Petri dishes (95 mm in diameter) were employed for the test
25 ml of the medium Nutrient-agar was inoculated with 0.1 ml of each 24 hours old culture of bacteria and 48 hours old cultures of yeast having predecided transmittance. Plates were kept on the levelled surface. Eight cups were bored in each plate after setting of the agar. Each cup was filled with 0.05 ml solution of the ester and was incubated at 32°C and 22°C respectively. The results were recorded after incubation period of 18 hours for bacterial and 48 hours for the yeast cultures.

The comparative inhibiting action of Ch E₁ to Ch E₇ against selected organisms is presented in Table XIX, where effectiveness is expressed in terms of inhibition zones measured in mm (at acidic and alkaline pH). The inhibiting influence of Ch E₁ to Ch E₇ when tested against Gram-positive organisms (1-4) at pH 4.5 (Chart 9), compounds Ch E₂, Ch E₆ and Ch E₅ exhibited maximum inhibition, even higher than that of chloramphenicol and chloramphenicol palmitate, followed by Ch E₁, Ch E₇ and Ch E₃, which showed inhibition higher than chloramphenicol palmitate against majority of organisms. Ch E₄ is found to be the least effective amongst all compounds. The extent of inhibition of Ch E₁ to Ch E₇ against various Gram-positive organisms was found to be of varied order. The inhibitory action of these compounds against Gram-negative organisms (5 to 8) showed
Relative 'in vitro' activity of new chloramphenicol esters Ch E₁ to Ch E₇ in acidic pH 4.5 against selected Gram positive, Gram negative and Yeast organisms, expressed as > or < that of either of two compounds of comparison.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch E₁ &lt; Cp</td>
<td>Ch E₁ &gt; Cp</td>
<td>Ch E₁ &gt; Cp</td>
<td>Ch E₁ &gt; Cp</td>
<td>Ch E₁ &gt; Cp</td>
</tr>
<tr>
<td>Ch E₂ &gt; Ch</td>
<td>Ch E₂ &gt; Ch</td>
<td>Ch E₂ &gt; Ch</td>
<td>Ch E₂ &gt; Ch</td>
<td>Ch E₂ &gt; Ch</td>
</tr>
<tr>
<td>Ch E₃ &lt; Cp</td>
<td>Ch E₃ &lt; Cp</td>
<td>Ch E₃ &lt; Cp</td>
<td>Ch E₃ &lt; Cp</td>
<td>Ch E₃ &lt; Cp</td>
</tr>
<tr>
<td>Ch E₇ &lt; Cp</td>
<td>Ch E₇ &lt; Cp</td>
<td>Ch E₇ &lt; Cp</td>
<td>Ch E₇ &lt; Cp</td>
<td>Ch E₇ &lt; Cp</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch E₁ &lt; Cp</td>
<td>Ch E₁, Ch E₇ &lt; Cp</td>
<td>Ch E₁ &lt; Cp</td>
<td>Ch E₁ &lt; Cp</td>
<td>Ch E₁ &lt; Cp</td>
</tr>
<tr>
<td>Ch E₂ &gt; Ch</td>
<td>Ch E₂ &lt; Cp</td>
<td>Ch E₂ &lt; Cp</td>
<td>Ch E₂ &lt; Cp</td>
<td>Ch E₂ &lt; Cp</td>
</tr>
<tr>
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<td>Ch E₇ &lt; Cp</td>
<td>Ch E₇ &lt; Cp</td>
<td>Ch E₇ &lt; Cp</td>
<td>Ch E₇ &lt; Cp</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch E₁ &lt; Cp</td>
<td>Ch E₁ &gt; Cp</td>
<td>Ch E₁ &gt; Cp</td>
<td>Ch E₁ &gt; Cp</td>
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<tr>
<td>Ch E₂ &gt; Cp</td>
<td>Ch E₂ &lt; Cp</td>
<td>Ch E₂ &lt; Cp</td>
<td>Ch E₂ &lt; Cp</td>
</tr>
<tr>
<td>Ch E₇ &gt; Cp</td>
<td>Ch E₇ &lt; Cp</td>
<td>Ch E₇ &lt; Cp</td>
<td>Ch E₇ &lt; Cp</td>
</tr>
</tbody>
</table>

Ch = Chloramphenicol, Cp = Chloramphenicol Palmitate
the superiority of Ch E₂, showing more effectiveness than that of chloramphenicol and chloramphenicol palmitate, while Ch E₅ and Ch E₆ were found superior to chloramphenicol palmitate. Ch E₁ and Ch E₇ were found either equal to or slightly inferior to chloramphenicol palmitate against the selected organisms, but Ch E₄ and Ch E₃ were found inferior. Ch E₂ has shown again its superiority when tested against yeast (9, 10 & 11). Compound Ch E₇ was found inferior than Ch E₂, while remaining compounds were found less effective than chloramphenicol palmitate.

Ch E₁ to Ch E₇, when tested against same organisms at alkaline pH (Chart 10), Ch E₇ and Ch E₃ showed inhibition higher than that of chloramphenicol palmitate and chloramphenicol against Gram-positive organisms. Effectiveness of Ch E₅, Ch E₁, Ch E₆ and Ch E₇ were found of descending order against same organisms while Ch E₄ exhibited minimum efficiency.

Ch E₇ and Ch E₃ were found superior to chloramphenicol and chloramphenicol palmitate against selected Gram-negative organisms. Effectiveness of Ch E₁ and Ch E₄ were inferior to Ch E₇ and Ch E₃, while remaining compounds exhibited inhibitory activity less than that of chloramphenicol.
Relative 'in vitro' activity of new chloramphenicol esters

<table>
<thead>
<tr>
<th>Gram positive</th>
<th>Gram negative</th>
<th>Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Bacillus pumilus NCTC 8241</strong></td>
<td><strong>5. Klebsiella pneumoniae ATCC 10031</strong></td>
<td><strong>9. Saccharomyces cerevisiae 3095</strong></td>
</tr>
<tr>
<td>Ch E₁ &gt; Ch E₁ &gt; Ch E₇ &gt; Ch E₄ &gt; Ch E₅ &gt; Ch E₆ &gt; Ch E₇ &gt; Ch</td>
<td>Ch E₁ &gt; Ch E₂ &gt; Ch E₃ &gt; Ch E₄ &gt; Ch E₅ &gt; Ch E₆ &gt; Ch E₇ &gt; Ch</td>
<td>Ch E₁ &gt; Ch E₂ &gt; Ch E₃ &gt; Ch E₄ &gt; Ch E₅ &gt; Ch E₆ &gt; Ch E₇ &gt; Ch</td>
</tr>
<tr>
<td><strong>2. Staphylococcus Aureus ATCC 6538P</strong></td>
<td><strong>6. Pseudomonas aeruginosa IFO 3898</strong></td>
<td><strong>10. Saccharomyces carlsbergensis 4228 ATCC 9080</strong></td>
</tr>
<tr>
<td>Ch E₁ &gt; Ch E₂ &gt; Ch E₃ &gt; Ch E₄ &gt; Ch E₅ &gt; Ch E₆ &gt; Ch E₇ &gt; Ch</td>
<td>Ch E₁ &gt; Ch E₂ &gt; Ch E₃ &gt; Ch E₄ &gt; Ch E₅ &gt; Ch E₆ &gt; Ch E₇ &gt; Ch</td>
<td>Ch E₁ &gt; Ch E₂ &gt; Ch E₃ &gt; Ch E₄ &gt; Ch E₅ &gt; Ch E₆ &gt; Ch E₇ &gt; Ch</td>
</tr>
<tr>
<td>Ch E₁ &lt; Ch E₂ &gt; Ch E₃ &gt; Ch E₄ &gt; Ch E₅ &gt; Ch E₆ &gt; Ch E₇ &gt; Ch</td>
<td>Ch E₁ &lt; Ch E₂ &gt; Ch E₃ &gt; Ch E₄ &gt; Ch E₅ &gt; Ch E₆ &gt; Ch E₇ &gt; Ch</td>
<td>Ch E₁ &gt; Ch E₂ &gt; Ch E₃ &gt; Ch E₄ &gt; Ch E₅ &gt; Ch E₆ &gt; Ch E₇ &gt; Ch</td>
</tr>
<tr>
<td><strong>4. Micrococcus flavus ATCC 10240</strong></td>
<td><strong>8. Escherichia coli ATCC 10536</strong></td>
<td></td>
</tr>
<tr>
<td>Ch E₁ &gt; Ch E₂ &gt; Ch E₃ &gt; Ch E₄ &gt; Ch E₅ &gt; Ch E₆ &gt; Ch E₇ &gt; Ch</td>
<td>Ch E₁ &gt; Ch E₂ &gt; Ch E₃ &gt; Ch E₄ &gt; Ch E₅ &gt; Ch E₆ &gt; Ch E₇ &gt; Ch</td>
<td></td>
</tr>
</tbody>
</table>

Ch = Chloramphenicol  
Cp = Chloramphenicol Palmitate
palmitate and chloramphenicol against all selected organisms.

Compounds Ch $E_7$ and Ch $E_3$ retained the same effectiveness against selected yeast ($9, 10$ and $11$). Remaining compounds Ch $E_1$, Ch $E_4$, Ch $E_6$ and Ch $E_2$ showed effectiveness against yeast in descending order of activity, while Ch $E_5$ was found to be completely ineffective against yeast in alkaline pH.
TOXICOLOGICAL STUDIES.
All the new chloramphenicol esters have shown promising antimicrobial activity. It is worthwhile to study its toxicity before proceeding to bioavailability studies. In the present investigations toxicity was carried out by using Karbar method (1937)\textsuperscript{53}. Toxicity was conducted on inbred swiss albino mice weighing between 18 to 25 g. Suspension of the compounds (Ch E\textsubscript{1} to Ch E\textsubscript{7}) was prepared with 0.1% acacia solution and given orally by blunt needle. Mice were observed for 48 hours. Each group consists of 5 mice. Compounds were tolerated upto 4 g/kg. Hence, LD\textsubscript{50} was not carried out by oral route. In the next set up of experiment LD\textsubscript{50} of the compounds were carried out by intra-peritoneal (i.p) route. Solution of the compounds was prepared in propylene glycol. Seven groups each containing 8 mice were used for determining LD\textsubscript{50} of each compound. After i.p. injection, mortality of mice was noted and results are tabulated of each compound. LD\textsubscript{50} of each compound was
LD<sub>50</sub> of compounds Ch E<sub>1</sub> to Ch E<sub>7</sub> and chloramphenicol palmitate was found to be 261.25, 261.25, 320.63, 280.00, 383.13, 280.00, 320.63 and 300 mg/kg respectively. It is concluded that new chloramphenicol esters under investigation were not more toxic than chloramphenicol palmitate. The LD<sub>50</sub> of each compound is represented in Tables XX to XXVI.
### TABLE - XX

**LD$_{50}$ OF THE COMPOUND Ch E$_1$ IN MICE (i.p.)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Dose difference</th>
<th>Dead</th>
<th>Mean **</th>
<th>Product @</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>500</td>
<td>50</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>450</td>
<td>150</td>
<td>9</td>
<td>8</td>
<td>400</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>100</td>
<td>7</td>
<td>5.5</td>
<td>825</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>100</td>
<td>4</td>
<td>2.5</td>
<td>250</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>70</td>
<td>1</td>
<td>0.5</td>
<td>35</td>
</tr>
<tr>
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<tr>
<td>7</td>
<td>15</td>
<td></td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Eight animals in each group. The apparent least dose lethal to all of a group is 450 mg/kg.

** The interval mean of the number of dead.

@ The product of the interval mean and the dose.

\[ \text{LD}_{50} = 450 - \left( \frac{1510}{6} \right) \]

\[ = 261.25 \text{ mg/kg} \]
<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Dose difference</th>
<th>Dead</th>
<th>Mean **</th>
<th>Product @</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>500</td>
<td>50</td>
<td>9</td>
<td></td>
<td></td>
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<tr>
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<td>825</td>
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<td>2.5</td>
<td>250</td>
</tr>
<tr>
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<td>100</td>
<td>70</td>
<td>1</td>
<td>0.5</td>
<td>35</td>
</tr>
<tr>
<td>6</td>
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<tr>
<td>7</td>
<td>15</td>
<td></td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ \text{LD}_{50} = 450 - \left( \frac{1510}{8} \right) \]

\[ = 261.25 \text{ mg/kg}. \]

* Eight animals in each group. The apparent least dose lethal to all of a group in 450 mg/kg.

** The interval mean of the number of dead.

@ The product of the interval mean and the dose.
<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Dose difference</th>
<th>Dead</th>
<th>Mean **</th>
<th>Product @</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>450</td>
<td>150</td>
<td>8</td>
<td>6.5</td>
<td>325</td>
</tr>
<tr>
<td>3</td>
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<td>100</td>
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<td>3.5</td>
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<td>1.5</td>
<td>150</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>70</td>
<td>1</td>
<td>0.5</td>
<td>35</td>
</tr>
<tr>
<td>6</td>
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<td>7</td>
<td>15</td>
<td></td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Eight animals in each group. The apparent least dose lethal to all of a group is 450 mg/kg.

** The interval mean of the number of dead.

@ The product of the interval mean and the dose.

\[ \text{LD}_{50} = 450 - \left( \frac{1035}{8} \right) \]

\[ = 320.63 \text{ mg/kg}. \]
### TABLE - XXIII

**LD$_{50}$ OF THE COMPOUND Ch E$_4$ IN MICE (i.p.)**

<table>
<thead>
<tr>
<th>Group*</th>
<th>Dose (mg/kg.)</th>
<th>Dose difference</th>
<th>Dead</th>
<th>Mean**</th>
<th>Product @</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
</tr>
<tr>
<td>2</td>
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<td>150</td>
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<td>375</td>
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<td>100</td>
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<td>5.0</td>
<td>750</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>100</td>
<td>4</td>
<td>2.0</td>
<td>200</td>
</tr>
<tr>
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<td>0.5</td>
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<tr>
<td>7</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1360</td>
<td></td>
</tr>
</tbody>
</table>

* Eight animals in each group. The apparent least dose lethal to all of a group is 450 mg/kg.

** The interval mean of the number of dead.

@ The product of the interval mean and the dose.

$. \quad \text{ID}_{50} = 450 - \left( \frac{1360}{8} \right)$

$\quad = 280 \text{ mg/kg.}$
TABLE - XXIV

\( \text{LD}_{50} \) OF THE COMPOUND Ch E\textsubscript{5} IN MICE (i.p.)

<table>
<thead>
<tr>
<th>Group*</th>
<th>Dose (mg/kg.)</th>
<th>Dose difference</th>
<th>Dead</th>
<th>Mean**</th>
<th>Product @</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>50</td>
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</tr>
<tr>
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<tr>
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<tr>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

* Eight animals in each group. The apparent least dose lethal to all of a group is 500 mg/kg.

** The interval mean of the number of dead.

@ The product of the interval mean and the dose.

\[ \text{LD}_{50} = 500 - \left( \frac{935}{8} \right) \]

\[ = 383.13 \text{ mg/kg.} \]
TABLE – XXV

ID$_{50}$ OF THE COMPOUND CH$_6$ IN MICE ( i.p.)

<table>
<thead>
<tr>
<th>Group*</th>
<th>Dose (mg/kg)</th>
<th>Dose difference.</th>
<th>Dead</th>
<th>Mean**</th>
<th>Product @</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>50</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>450</td>
<td>150</td>
<td>9</td>
<td>7.5</td>
<td>375</td>
</tr>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

* Eight animals in each group. The apparent least dose lethal to all of a group is 450 mg/kg.

** The interval mean of the number of dead.

@ The product of the interval mean and the dose.

\[ \text{ID}_{50} = 450 - \left( \frac{1360}{8} \right) \]

\[ = 280.00 \text{ mg/kg}. \]
**TABLE - XXVI**

\[LD_{50}\] OF THE COMPOUND Ch7 IN MICE (i.p.).

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Dose difference</th>
<th>Dead</th>
<th>Mean**</th>
<th>Product®</th>
</tr>
</thead>
<tbody>
<tr>
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<td>50</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>450</td>
<td>150</td>
<td>8</td>
<td>6.5</td>
<td>325</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>100</td>
<td>5</td>
<td>3.5</td>
<td>525</td>
</tr>
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<td>0.5</td>
<td>35</td>
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<tr>
<td>6</td>
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<td>15</td>
<td></td>
<td>0</td>
<td></td>
<td>1035</td>
</tr>
</tbody>
</table>

* Eight animals in each group. The apparent least dose lethal to all of a group is 450 mg/kg.

** The interval mean of the number of dead.

@ The product of the interval mean and the dose.

\[LD_{50} = 450 - \frac{1035}{8}\]

\[= 320.63 \text{ mg/kg.}\]
The broad definition of biopharmaceutics would include drug latentation and the preparation of different salts of a given acidic or basic drug for the purpose of altering the biological effects elicited by the parent drug. Hence, it encompasses the study of the relation between the nature and intensity of the biological effects observed in animals and man.

The following factors affect the bioavailability of drugs:

1) Simple chemical modification of drugs such as formation of esters, salts and complexes.

2) Modification of the physical state, particle size and/or surface area of the drug available to the adsorption sites.
3) Presence or absence of the adjuvants in the dosage form with the drug.

4) The type of dosage form.

5) The pharmaceutical process or processes by which the dosage form is manufactured.

In the present investigations, simple modification of chloramphenicol is carried out by synthesizing its esters.

For the primary bioavailability studies suspension of esters were prepared in propylene glycol and blood serum level of chloramphenicol was investigated in comparison with equivalent dose of chloramphenicol and chloramphenicol palmitate.

**BIOAVAILABILITY IN RABBITS:**

Preliminary bioavailability studies of compounds were carried out on rabbits weighing 1.8 - 2.3 kg. They were fasted for 16 - 18 hours prior to the experiment. Blood samples were collected from marginal ear vein. The suspensions of compounds were prepared in propylene glycol and administered orally at a dose level of 150 mg/kg body weight. Blood samples were collected after 2, 4, 6, 8, 10 and 12 hours after administration of the compounds.
Blood samples were centrifuged and serum was separated. Chloramphenicol in serum (mcg/ml) was estimated by microbiological method using Sarcina lutea ATCC 9341.

Four rabbits were used for testing one compound.

Average value and standard error were calculated and results are tabulated in Table XXVII.

**BIOAVAILABILITY IN DOGS:**

Dogs weighing between 10 to 15 kg were used. They were fasted for 18 hours prior to the experiment. Compounds were administered orally through a mouth tube at a dose level of 150 mg/kg (equivalent of chloramphenicol). Blood samples were withdrawn from the artery at the interval of 2, 4, 6, 8, 10 and 12 hours. Serum was separated by centrifuging each sample. Chloramphenicol in serum was estimated by microbiological method using Sarcina lutea ATCC 9341. Minimum three dogs were used for testing each compound. Average value and standard error was calculated. The results are recorded in Table XXVIII.

**DETERMINATION OF BIOAVAILABILITY:**

The Wagner's one compartmental method was used for determination of bioavailability of compounds. The area
under the curve was calculated by counting the number of squares present under the curve. The formula used for calculating fraction of dose absorbed was \( F = \frac{0.693 A \cdot V}{T \cdot D} \).

The highest factor obtained was considered as 100% availability and comparative availability was calculated from it.

**MICROBIOLOGICAL TESTING:**

**TEST ORGANISM:** Sarcina lutea A.T.C.C. 9341

**MAINTENANCE MEDIUM:** (for slant and Roux bottle)

- Peptone: 6.0 g
- Casitone: 4.0 g
- Yeast extract: 3.0 g
- Beef extract: 1.5 g
- Dextrose: 1.0 g
- Distilled water: 1000 ml
- Agar: 20.0 g
- pH adjusted to 6.6
- Sterilization: 15 minutes sterilization at 15 lb/sq inch (121°C).

**ASSAY MEDIUM:**

a) Base layer: Same as maintenance medium.

b) Seed layer: Same as base layer assay medium. (amount of agar was reduced to 1.0% instead of 2.0%).
PHOSPHATE BUFFER:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Monopotassium dihydrogen phosphate</td>
<td>8.0 g</td>
</tr>
<tr>
<td>Distilled water to make</td>
<td>1000 ml</td>
</tr>
<tr>
<td>pH adjusted to</td>
<td>6.00</td>
</tr>
<tr>
<td>Sterilization</td>
<td></td>
</tr>
</tbody>
</table>

15 minutes sterilization at 15 lb/sq inch (121°C).

INOCULUM:

From a fresh slant of test organism a loopful of cells were transferred into 10 ml of sterile 0.9% normal saline solution to prepare a suspension. A portion of suspension was transferred to maintenance medium in Roux bottle aseptically. Inoculated Roux bottles were incubated at 32°C for 18 to 24 hours. The growth was harvested aseptically in 0.9% normal saline solution. The suspension was stored at 4°C and used within two weeks period.

ASSAY PROCEDURE:

PREPARATION OF STANDARD SOLUTIONS:

The working standard was weighed accurately and diluted with phosphate buffer (pH 6.0) so as to have antibiotic 100 mcg/ml. From this dilution following five dilutions were prepared.
A : 3.2 ml diluted to 10 ml (32 mcg/ml)
B : 4.0 ml diluted to 10 ml (40 mcg/ml)
C : 5.0 ml diluted to 10 ml (50 mcg/ml)
D : 6.25 ml diluted to 10 ml (62.5 mcg/ml)
E : 7.81 ml diluted to 10 ml (78.1 mcg/ml)

(Standard solution 'C' is also known as 'Reference Unit')

PREPARATION OF SAMPLE SOLUTION:
Dissolved completely, the extracted material in exactly 1.0 ml of phosphate buffer (pH 6.0) and made a uniform solution.

PREPARATION OF PLATES:
12 Petri plates were employed for standard solutions and 3 plates for each sample solution. 21 ml of uninoculated base layer assay medium was poured in each plate and allowed to solidify. 4.0 ml of seed layer assay medium inoculated with the test organism was added in each plate. Allowed it to solidify. Six cups were prepared in each plate, each having a diameter of 6 mm.

SETTING OF ASSAY:
The assay was put in triplicate as follows. Made four sets,
each of three plates for standard solutions, and one set of three plates for each sample solution. Placed a mark from the beneath to a cup of each plate. 0.1 ml of standard solution and sample solution pipetted in their respective cups.

STANDARD:
In each plate 0.1 ml of the following dilutions was placed alternatively.

Set No.1 standard solution A and standard solution C.
Set No.2 standard solution B and standard solution C.
Set No.3 standard solution D and standard solution C.
Set No.4 standard solution E and standard solution C.

SAMPLE:
In each plate, pipetted 0.1 ml of following dilutions alternatively.

Sample solution and standard solution C.

Allowed all the solutions to diffuse well at R.T. for 30 minutes covered each plate with a filter paper and a lid.

INCUBATION PERIOD:
Incubated all the plates at 32°C for 18 hours.
ZONE MEASUREMENT:

Measured the diameter of zone of inhibition on zone reader instrument.

CALCULATION:

The average of all the nine readings of each level of standard solution was calculated (individual average of A, B, D, E and Ac, Bc, Dc and Ec). Found out other average (an average of reference units) of standard solution C zone diameter (i.e., an average of Ac, Bc, Dc and Ec). To find out the 'correction factor' subtracted each individual reference unit average (individual average of Ac, Bc, Dc and Ec) from 'an average of reference unit zone diameter'. Added this 'standard solution zone diameter' (average of A, B, D and E) to get 'corrected average'.

Calculated zone diameter at the highest concentration (YH) and calculated zone diameter at the lowest concentration (YL) with the help of following formula.

\[
YH = \frac{3(E) + 2(D) + C - A}{5}
\]

\[
YL = \frac{3(A) + 2(B) + C - E}{5}
\]
The graphs of these YH and YL values against their respective standard concentration were plotted.

The average of nine readings of 'sample' (Yu) and average of nine readings of standard solutions (Ys) from the set of 'sample' were calculated. The value of (Yu - Ys) was added to the value to a 'reference unit' zone diameter from the graph. Read the equivalent amount to standard from the graph with the help of above value (i.e. reference unit zone on graph + Yu - Ys). The content of chloramphenicol was calculated.

Content = equivalent amount to standard from graph x \( \frac{\text{standard factor}}{100} \) x dilution x conversion.

Wagner method was adopted to calculate the fraction of drug absorbed in dogs.

\[
F = \frac{0.693 \times A \times V}{T_{1/2} \times D}
\]

Where:

- \( F \) = Fraction of drug absorbed
- \( A \) = Area under concentration curve from zero to infinite time.
- \( V \) = Volume of fluid of animal body (average volume of the blood of a dog is taken as 2 litres).
- \( T_{1/2} \) = Half life of chloramphenicol - 2.5 hours.
\[ D = \text{Dose of drug equivalent to chloramphenicol concentration (mcg/ml).} \]

Calculation based on chloramphenicol content of each ester.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Calculation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>( F = \frac{0.693 \times 7.09 \times 2000 \times 100}{2.5 \times 109410} )</td>
<td>= 3.592%</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>( F = \frac{0.693 \times 26.79 \times 2000 \times 100}{2.5 \times 84300} )</td>
<td>= 17.610%</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>( F = \frac{0.693 \times 7.184 \times 2000 \times 100}{2.5 \times 105765} )</td>
<td>= 3.765%</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>( F = \frac{0.693 \times 16.194 \times 2000 \times 100}{2.5 \times 109660} )</td>
<td>= 8.188%</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>( F = \frac{0.693 \times 7.463 \times 2000 \times 100}{2.5 \times 100140} )</td>
<td>= 4.315%</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>( F = \frac{0.693 \times 8.805 \times 2000 \times 100}{2.5 \times 113060} )</td>
<td>= 4.317%</td>
<td></td>
</tr>
</tbody>
</table>
TABLE XXIX

TABLE SHOWING COMPARATIVE CHLORAMPHENICOL CONTENT AND PERCENTAGE FRACTION ABSORBED OF EACH COMPOUND IN DOGS.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chloramphenicol content in 150 mg of compound.</th>
<th>Fraction of chloramphenicol absorbed</th>
<th>Comparative availability.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch E₁</td>
<td>109.410</td>
<td>3.592</td>
<td>20.40</td>
</tr>
<tr>
<td>Ch E₂</td>
<td>84.300</td>
<td>17.610</td>
<td>100.00</td>
</tr>
<tr>
<td>Ch E₃</td>
<td>105.765</td>
<td>3.765</td>
<td>21.39</td>
</tr>
<tr>
<td>Ch E₄</td>
<td>109.660</td>
<td>8.188</td>
<td>46.52</td>
</tr>
<tr>
<td>Ch E₅</td>
<td>100.140</td>
<td>4.315</td>
<td>24.51</td>
</tr>
<tr>
<td>Ch E₆</td>
<td>113.060</td>
<td>4.317</td>
<td>24.52</td>
</tr>
<tr>
<td>Ch E₇</td>
<td>66.760</td>
<td>6.816</td>
<td>38.72</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>106.000</td>
<td>7.650</td>
<td>43.46</td>
</tr>
<tr>
<td>Chloramphenicol Palmitate</td>
<td>86.320</td>
<td>9.777</td>
<td>55.55</td>
</tr>
</tbody>
</table>
SERUM LEVELS IN DOGS AFTER ORAL ADMINISTRATION OF NEWLY SYNTHESIZED CHLORAMPHENICOL ESTERS.

Chloramphenicol is rapidly absorbed from the gastrointestinal tract, significant plasma concentrations are obtained within 30 minutes and peak values are reached in approximately 2 hours. Chloramphenicol palmitate produces sustain plasma concentration level. The bioavailability of newly synthesized chloramphenicol esters is summarised in the table XXIX. From these biological values, the structural relationship and serum level of chloramphenicol can be interpreted. These biological values in animals are important guide line for evaluating in human beings.

In the general structure of aroyl esters of chloramphenicol (compounds Ch E₁ to Ch E₅), various substituents at R and R₁, reflect the bioavailability of the compounds. In compound Ch E₁ with R = OH and R₁ = H, the peak blood level (28 ± 3.852 mcg/ml) is obtained at 2 hours and therapeutic blood level is maintained upto 8 hours. By substituting acetyl group at R and keeping R₁ = H (Ch E₂) a notable serum level was observed. The peak level (83.30 ± 3.531) was observed at 2 hours and therapeutic level is maintained upto 14 hours. In compound Ch E₃, R was kept constant and R₁ is substituted by amino group. The peak serum level (24 ± 2.0 mcg/ml) was obtained
after 2 hours and therapeutic level was observed up to 8-10 hours. Thus, OH group at R and amino group at \( R_1 \) in Ch \( E_3 \), decreases the availability of chloramphenicol. By substituting R by H and \( R_1 \) by \( NH_2 \) group in compound Ch \( E_4 \) enhances the bioavailability of the compound. The peak serum level \((61.66 \pm 4.417)\) was obtained after 4 hours and therapeutic blood level was observed up to 12 hours. Thus, by replacing OH group by 'H' at R increases the peak level and duration of chloramphenicol levels in blood, but acetylation on \( NH_2 \) group in compound Ch \( E_5 \) decreases the peak level \((29 \pm 3.292)\) and therapeutic blood level \((10 \text{ hours})\) of chloramphenicol. Bioavailability of pyridoyl chloramphenicol ester (Ch \( E_6 \)) and penam chloramphenicol ester (Ch \( E_7 \)) was investigated. The peak level of the latter is obtained after 6 hours \((34.66 \pm 2.614 \text{ mcg/ml})\) while with the first compound peak level \((36 \pm 3.057 \text{ mcg/ml})\) was obtained after 2 hours.

It can be concluded that substitutions of \( R = O.COOCH_3 \) with respect to \( R_1 = H \) and \( R = H \) with respect to \( R_1 = NH_2 \) might be highly absorbed from G.I. tract and maintains sustained serum level. Moreover, the same compounds have shown good antimicrobial activity against various microbes and fungus in vitro. High peak level and sustained serum level is
important criteria for preventing microbial resistance to the drug. Comparative bioavailability data (in dogs) of new chloramphenicol esters in comparison with chloramphenicol and chloramphenicol palmitate are presented in graphs 2 and 3.
COMPARATIVE BIOAVAILABILITY OF CHLORAMPHENICOL ESTERS
CHLORAMPHENICOL & CHLORAMPHENICOL PALMITATE IN DOGS

SERUM CONC. OF CHLORAMPHENICOL, mcg/mL

CHLORAMPHENICOL
CHLORAMPHENICOL PALMITATE
COMPOUND Ch E2
COMPOUND Ch E4

hours: 2 4 6 8 10 12 14 16
SERUM CONC. OF CHLORAMPHENICOL mg/ml.

hours: 2  4  6  8  10  12  14  16

CHLORAMPHENICOL
CHLORAMPHENICOL PALMITATE
COMPOUND E1
" E3
" E5
" E6
" E7