IN VITRO CHANGES AND RECOVERY OF ERYTHROCYTES SHAPE IN INDUCED ATHEROGENESIS IN RABBITS

T. M. VATSALA AND MEGHA SINGH

Bio-Medical Engineering Division, Indian Institute of Technology, Madras 600 036, *India*

CHANGES in arterial wall during atherogenesis have been reported[1, 2]. The major emphasis has been given to the alterations induced by hyperlipidemia at the vari- -tes of these vessels such as bend, branching, , etc. But little emphasis has been given -ape of erythrocytes during atherogenesis. -olesterol levels in plasma results in the - of target and spur cells-acanthocytes[3–5]. -rence of acanthocytes were also observed -ain liver diseases and in familial abetalipo--emia where there is an increase in plasma -erol levels[4–6]. Recently it was reported that the erythrocytes of guinea pigs, fed with cholesterol-rich diet, were irregularly shaped with asymmetrical spicules on their surfaces[7]. During atherogenesis the red cells remain in high cholesterol medium throughout their life span, wherein, cholesterol would tend to stagnate and accumulate in the red cells and this may result in the formation of acanthocytes. We have observed acanthocytes with increased tendency of aggregation associated with a change in cellular area in rabbits with high plasma cholesterol levels (1000–1400 mg %). The erythrocytes shape of another group of rabbits with comparable plasma cholesterol levels, when fed with water soluble extract of 20 g fresh onion[8] along with atherogenic diet, remains normal, compared to normal control erythrocytes. This may be due to the presence of some factors in plasma from onion extract which counterbalances the effect of high cholesterol on the erythrocyte membrane. To observe the effects of various factors which may contribute toward the alteration and restoration of normal shape during atherogenesis, the erythrocytes were obtained from normal and atherosclerotic rabbits and were studied in *in vitro*.

White albino rabbits of same age, sex and body weight (800–1000 g) were fed with a normal laboratory diet and were divided into three groups (10 animals in each group) : Group I served as normal controls; Group II animals were fed with normal diet plus 0·5% cholesterol (atherogenic diet) and Group III animals with water soluble extract of 20 g onion along with atherogenic diet. Rabbits with serum cholesterol levels between 1000–1400 mg % were taken up and these levels were maintained within the above range by the dietary adjustment of cholesterol. At the end of three months, blood was drawn by ear vein puncture into tubes containing ammonium oxalate (as anticoagulant). The blood samples were centrifuged and plasma was separated. The erythrocytes of each group were washed with isotonic saline (0·9%) three times and the supernatant layers were removed by centrifugation after each wash.

The washed cells from Group II were mixed with the plasma of Group I (set 1) and cells from Groups I and III were mixed with plasma from Group II (Sets 2 and 3 respectively). The volumes of fresh plasma and cells in each set were adjusted to the level of normal hematocrit (38%) and were incubated at 37° C for one hour. After incubation, smears were made on microscopic slides from each sample and were observed under Leitz microscope at 1000 ×.

The microphotographs are shown in Figures 1–4. Figure 1 (*a*, *b* and *c*) shows the erythrocytes from Group I, II and III animals. The erythrocytes from Groups I and III have normal shapes whereas from Group II show crenation (macroplania) and aggregation. Figure 2 (*a*, *b*) shows the erythrocytes of set 1 which have recovered from their altered shape and are comparable with normal control cells. The cells of set 2 show crenation and deformation associated with a decrease in cellular area [Figure 3 (*a*, *b*)]. Figure 4 (*a*, *b*) shows the cells of set 3. These cells are comparable with the cells of set 2. This indicates that the normal cells whether they are from normal control or onion plus cholesterol-fed animals, are being influenced by the higher cholesterol content of the plasma of Group II.

Biochemical analyses of the plasma samples were made for the determination of cholesterol[9] and fibrinogen[10] levels. Results are presented in Table I.

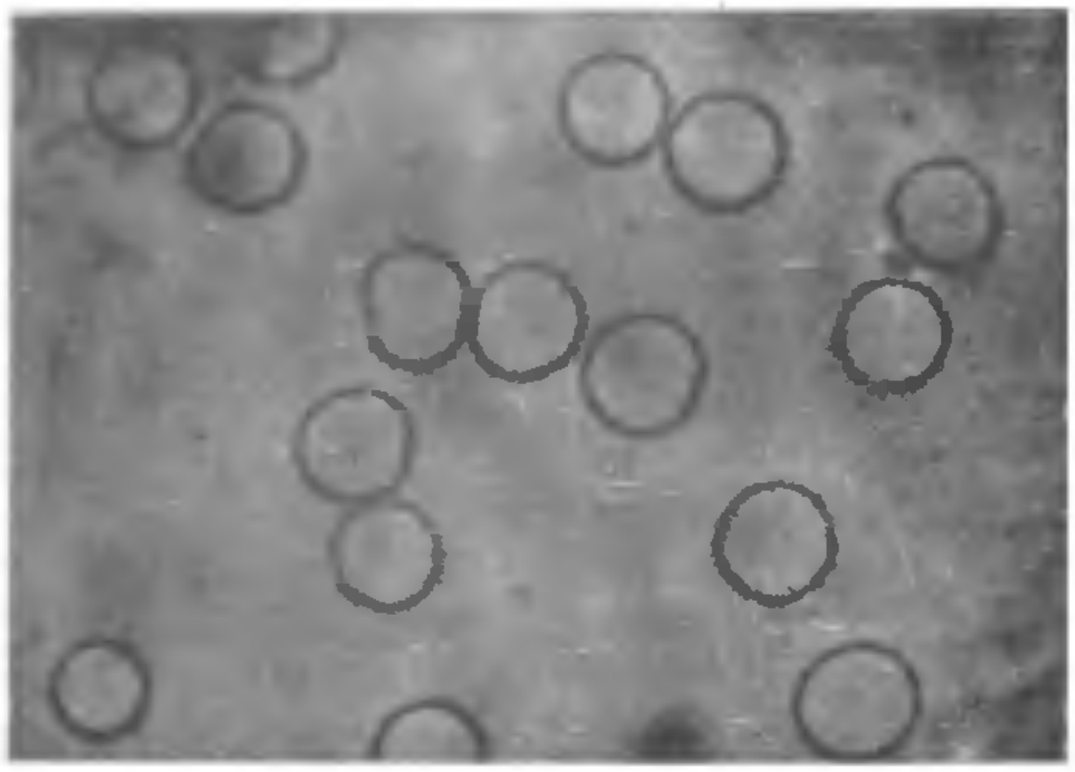
TABLE I

Levels of cholesterol and fibrinogen in various groups

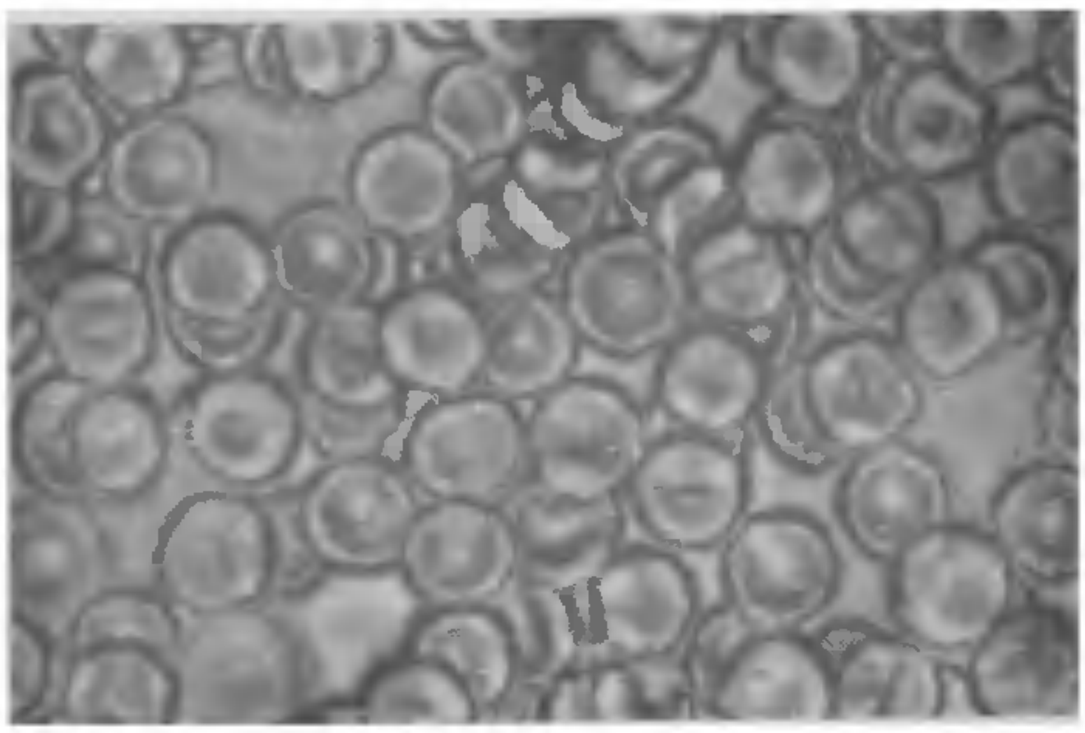
Group	Cholesterol mg %	Fibrinogen mg %
I	97·0 ± 10·35*	288 ± 15
II	1174·3 ± 267·50	620 ± 24
III	951·6 ± 105·40	600 ± 18

* Mean ± S.D.

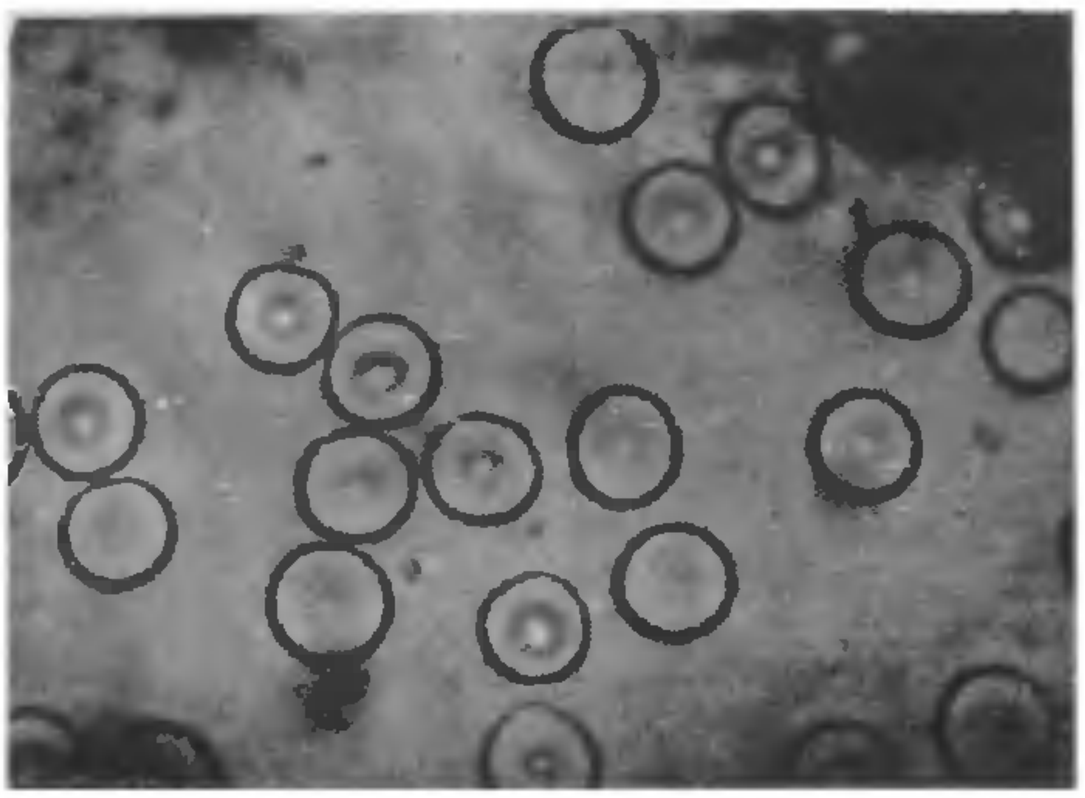
The typical behaviour of normal plasma for erythrocytes shape recovery may be due to the low levels of cholesterol and fibrinogen. The occurrence of similar phenomenon, *i.e.*, normal shape of erythrocytes in Group III and becoming crenated after being



(*a*)



(*b*)



(*c*)

FIG. 1 (*a*, *b*, *c*). Erythrocytes obtained from rabbits of various groups : (*a*) Group I (normal diet), (*b*) Group II (Normal diet plus 0·5% cholsterol) and (*c*) Group III (diet same as for Group II plus extract of 20 g fresh onions).

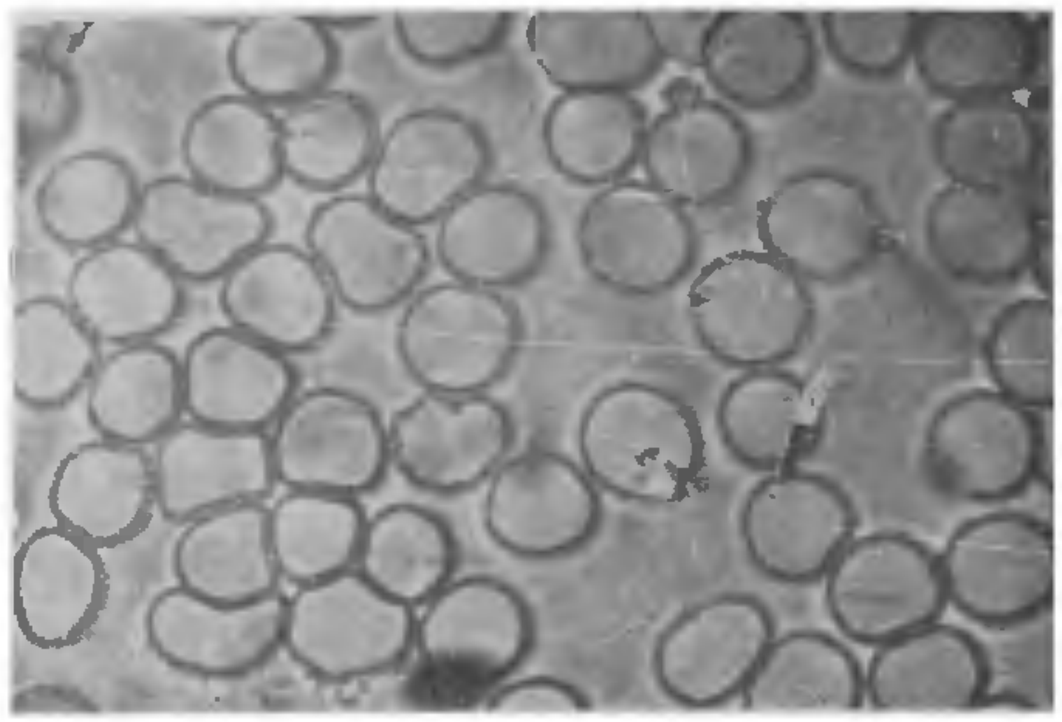


FIG. 2. Erythrocytes of Group II, after incubation with plasma of Group I, showing the recovery these cells from crenation.

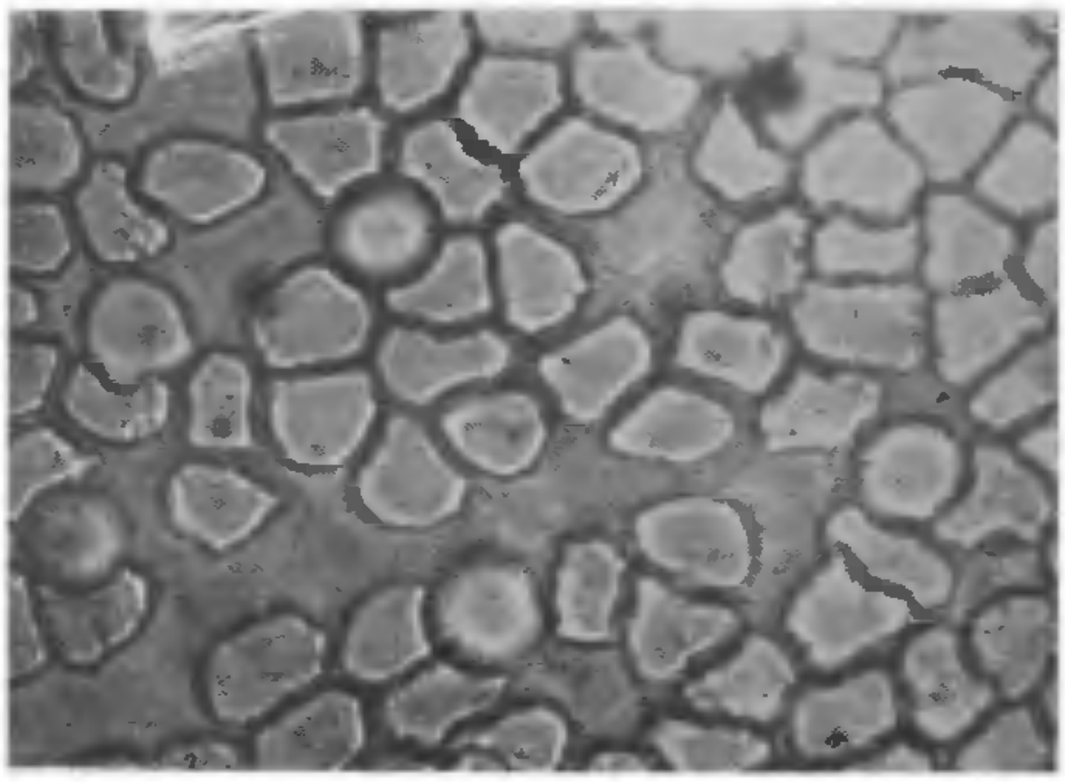


FIG. 3 Erythrocytes of Group I, after incubation with plasma of Group II, showing the various changes induced to the normal cells.

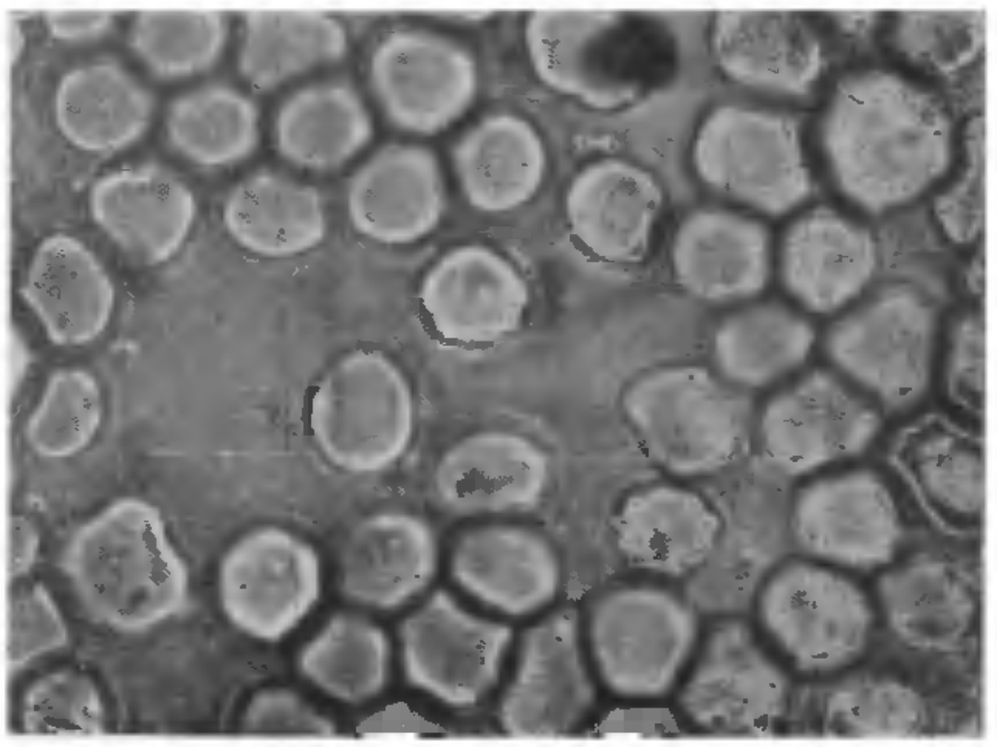


FIG. 4. Erythrocytes of Group III, after incubation with plasma of Group II, showing the various changes similar to Fig. 3.

subjected to Group II plasma, indicates the presence of some factors in onion extract which help for retaining the normal shape of erythrocytes. To observe the effect of Group III plasma on Group II erythrocytes, these were mixed and incubated by similar method as mentioned above. Crenation of the cells disappears and they closely resemble normal control cells, as shown in Fig. 5.

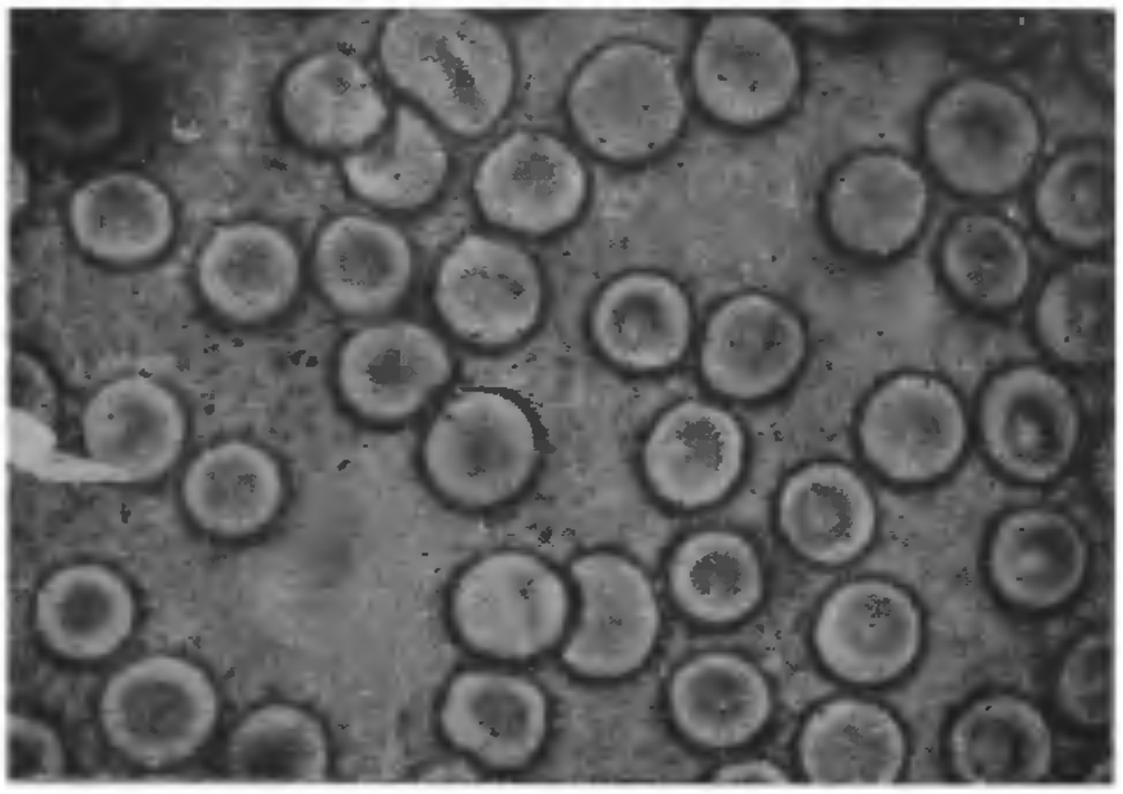


FIG. 5. Erythrocytes of Group II, after incubation with plasma of Group III, show the recovery from crenation and appear similar to normal control cells.

Recovery of the cells from crenation indicates the presence of some factors in Group III plasma which counterbalances the cholesterol effect and this plasma behaves similar to the normal control plasma.

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4-DIMETHYLAMINO PYRIDINE-1-OXIDE COMPLEXES OF LANTHANIDE PERCHLORATES

N. S. NAVANEETHAM AND S. SOUNDARARAJAN
Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore 560 012

ABSTRACT

Adducts of Lanthanide Perchlorates with 4-dimethyl amino pyridine-1-oxide (DMPO) have been synthesized for the first time and characterized by analysis, electrolytic conductance, infrared, proton NMR and electronic spectral data. The complexes have the compositions $Ln_2(DMPO)_{13}(ClO_4)_6$ (Ln = La, Pr, Nd and Sm) and $Ln(DMPO)_6(ClO_4)_3$ (Ln = Gd, Tb, Dy, Ho and Yb). A tentative coordination number of seven for the complexes of the type $Ln_2(DMPO)_{13}(ClO_4)_6$ and of six for the type $Ln(DMPO)_6(ClO_4)_3$ have been assigned.

2 INTRODUCTION

THE study of the coordination compounds of a variety of lanthanide salts, with pyridine-N-oxide (PyO) and methyl substituted pyridine-N-oxides has shown that the substitution of the methyl group at 3 and 4 positions of the PyO moiety has no influence on the coordination number around the lanthanide ions[1-3]. We have now initiated a systematic programme involving adducts of lanthanide salts with pyridine-1-oxides having substituents other than the methyl group. We report in this paper the preparation and characterization of the complexes of lanthanide perchlorates with 4-dimethylamino pridine-1-oxide with an attempt to compare the complexes with those of 4-MePyO[3], 4-chloro PyO and 4-nitro PyO[4]. The complexes have been characterized by analysis, conductance, IR, NMR and electronic spectra.

2. EXPERIMENTAL

2.1. *Preparation of the Ligand*

4-Nitro pyridine-1-oxide was prepared by nitration of pyridine-1-oxide as described by Katritzky[5]. 4-Chloropyridine-1-oxide was obtained by the reaction of 4-nitropyridine-1-oxide with acetyl chloride according to the method given by Ochiai[6]. 4-Dimethylamino-pyridine-1-oxide was now prepared by reacting