PHARMACOLOGICAL SCREENING FOR ANTI-INFLAMMATORY ACTIVITY IN A NOVEL SERIES OF COMPOUNDS BRIDGING STEROIDAL AND NON-STEROIDAL DRUGS.

BY

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A C K N O W L E D G E M E N T S

Dr. Gichuru Muriuki; My Supervisor

His energy seen only with Pharmacologists was very providing and so ensued My Interest in this Research Project was Maximal.

His Guidance in the Field of Pharmacology and in particular towards "Evaluation of New Drugs" gave me confidence with which I expatiate upon and so the Presentation!

Mr. Kamau; Lecturer in Pharmaceutical Chemistry

Who not only consummated every piece of this work but to who we all owe a lot for designing and developing the FK Series and so leading the Project.

Thru the FK's Mankind might be relieved off Inflammation!

The Technical Staff of the School of Pharmacy especially in Pharmacology Njoroge, Munenge (Mrs.), Wangai and Ochieng. For without that Group the work presented won't have been.

Finally to My Sister Mary Mbugua for Her Dexterous Secretarial Work!

D E D I C A T I O N S

To that Family which I belong,

MBUGUA and WAIRIMU

na ciana ciao

For Having Seen Me Thru ...

MAY YOU LIVE IN A LESS INFLAMMED WORLD!!

CONTENTS

PHARMACOLOGICAL SCREENING FOR ANTI-INFLAMMATORY EFFECTS IN A NOVEL SERIES OF COMPOUNDS BRIDGING STEROIDAL AND NON-STEROIDAL AGENTS:-

Key Words:- Carrageenan, Anti-inflammatory, steroidal, Non-steroidal, FKII-IND, FK12 AC-LACT, FK13 AC-CL.

ABSTRACT:

1. A Novel Series of Compounds bridging steroidal and Non-steroidal agents have been synthesized in the Department of Pharmacy, University of Nairobi and were screened for anti-inflammatory activity.

2. A standard Model, Inhibition of Carrageenan induced Rat Paw oedema was used with slight modifications.

3. Renal changes were assessed in Rats and were found to be comparable with agents used Clinically.

4. It was concluded that the compound FK13 AC-CL at low doses was more potent than Indomethacin. It is also possible that at Low doses the compounds have a similar mechanism of action to Non-steroidal Anti-inflammatory agents and at higher doses similar to steroidal anti-inflammatory Agents.

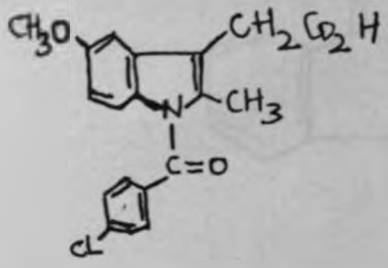
5. The usefulness of other pharmacological models like the Delayed Manifestation of Ultra Violet (UV) induced Erythema in Guinea-Pig in the assesment of mode of Action of these compounds, which is postulated to be via inhibition of PG Synthesis at two levels, is discussed.

INTRODUCTION

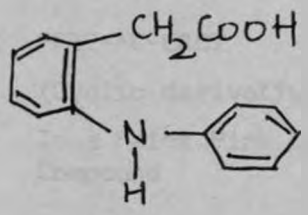
An ideal anti-inflammatory agent should be capable of reversing all Clinical Manifestations of inflammation namely Pain, Redness, Heat and Swelling. Clinically, useful anti-inflammatory agents are either steroidal or Non-steroidal structures.

In the design of the Compounds FKII-IND, FK12 AC-LACT, and FK13 AC-CL useful features found in the steroids and Non-steroidal anti-inflammatory drugs were bridged. Similarly undersirable effects usually encountered in the two classes were eliminated to a certain extent.

(I) Non-Steroidal Anti-inflammatory Agents (NSAID)

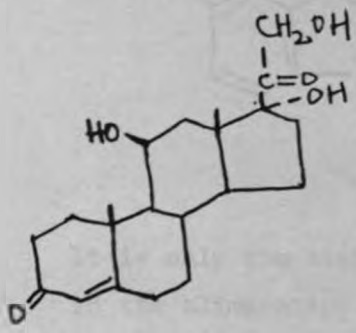


INDOMETHACIN

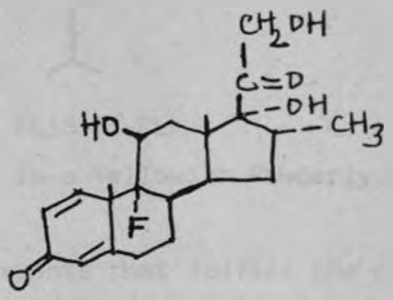


DICLOFENAC

(II) STEROIDAL ANTI-INFLAMMATORY AGENTS

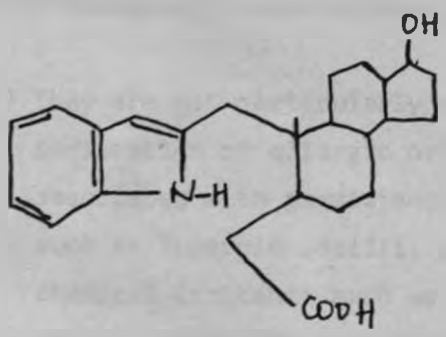


HYDROCORTISONE



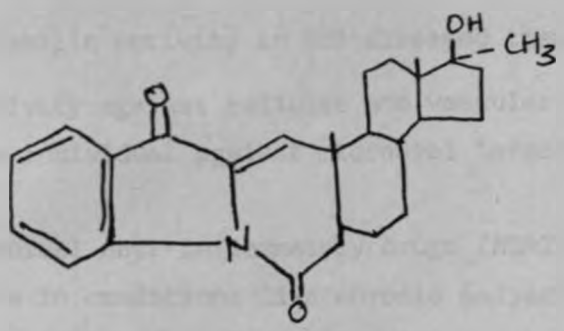
DEXAMETHASONE

(III) FK COMPOUNDS



FKII-IND

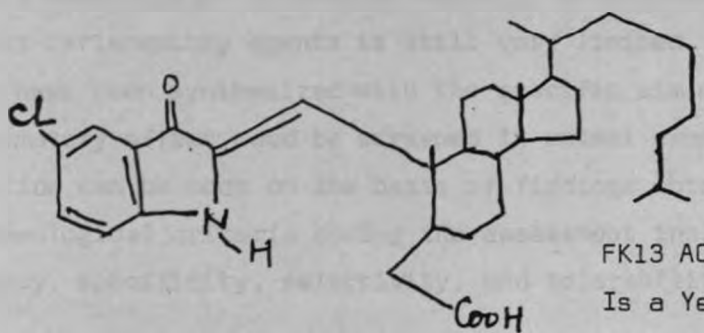
Is a dirty white Compound in powderly form.



FK12-AC-LACT

(Cyclic derivative, Prodrug?)

Is a Faint Pink powderly Compound



FK13 AC-CL

Is a Yellowish Powderly Compound

It is only the steroidal anti-inflammatory agents that fulfils the criteria in the elimination of Symptoms of inflammation. But their usefulness is limited by the fact that:-

(i) High doses of steroids are required for the complete suppression of subjective phenomena of inflammation like stiffness, soreness and tenderness and objective signs like joint swelling, flexion contractures and histopathological Features. This tends to bring about Life-threatening complications.

(ii) They are not particularly of Therapeutic benefit unless in inflamation of allergic origin. Infact they are harmful in conditions associated with persistence of viable pathogenic micro-organisms such as Tubercle bacilli, pyogenic bacteria or in conditions of chemical irritants such as the gastric juice. Tuberculosis and peptic ulcers are exacerabated and could be reactivated once healed.

(iii) Lower Host's resistance to microbial infection. Hence the increased incidences of Fungal, viral and Protozoal infections in patients receiving immuno suppressive doses of anti-inflammatory steroids.

During the search of steroids with useful anti-inflammatory effects attempts have been laid upon steroids devoid of:

(a) Metabolic activity in Non-diseased tissues

(b) Activity against cellular and vascular mechanisms which defend the individual against microbial infection.

Non-steroidal anti-inflammatory drugs (NSAID) are currently the drugs of choice in conditions like chronic polyarthritis and inflammations of non-allergic origin. The propensity to produce Gastro-intestinal bleeding and ulceration in Animals (1) and Human (2) has been an index for ascertaining toxicity of NSAID.

But the knowledge of Structural Activity Relationship (SAR) in the field of anti-inflammatory agents is still very limited. Therefore, substances which have been synthesized with the specific aim of achieving an anti-inflammatory effect need be screened in animal experiments, so that a selection can be made on the basis of findings obtained. The classical pharmacological criteria during the assessment includes activity, potency, efficacy, specificity, selectivity, and tolerability.

In studies on drug action it has been observed that the physico-chemical properties of the drug molecule plays an important role in determining the Activity, specificity and toxicity of the drug. This is true for Anti-inflammatory drugs where the acidity constant P^{Ka} have been implicated not only with the toxicity (3) but in the activity (4) of the NSAIDS.

The partition coefficient has been attributed to be the major cause for the maximal activity observed with Dexamethasone compared with other anti-inflammatory steroids. Infact steroids' efficacy is due to the high degree of Lipopholism as reflected by partition coefficient (LOG P) in n-octanol and aqueous buffer at P^{H} 7.4, Table I.

From preliminary studies (5), the FK series of compounds were found to have maximum activity against carrageenan induced Paw oedema when

P^{Ka} = 5 to 7

LOG P = 2 to 6

It is an observation which agrees with studies done with Diclofenac (4). The two physico-chemical factors are demonstrated in Table II where the two sets of drugs are compared.

TABLE I

COMPOUND	PARTITION COEFFICIENT LOG P	ANTI-INFLAMMATORY ACTIVITY
HYDROCORTISONE	0.89	1.0
CORTISONE	1.45	0.75
PREDNISOLONE	1.42	3.5
METHYLPREDNISOLONE	1.85	5.0
DEXAMETHASONE	1.90	190
PREDNISONE	1.46	4
BETAMETHASONE	1.98	70
9α-FLUOROCORTISONE	1.68	6
TRIAMCINOLONE	1.21	5

. The Anti-inflammatory activity is compared to the Lipophilicity of the Steroidal drug and it is observed that maximal anti-inflammatory activity at Dexamethasone corresponds with maximal lipophilicity.

TABLE II

Physico-chemical Properties of the FK Compounds compared with other Non-steroidal anti-inflammatory Agents. The P^{Ka} and LOG P were determined by the method by KOFI W.M. (24).

COMPOUND	ACIDITY CONSTANT P^{Ka}	PARTITION COEFFICIENT LOG P
FKII - IND		
FK12 AC - LACT	7.0	
FK13 AC-CL	6:56	5.31
Phenylbutazone	4.8	0.7
Mefanamic Acid	4.2	2.04
Indomethacin	4.2	1.0
Diclofenac	4.0	1.13

Infact in the development of Diclofenac it was thought essential that an acidic range was favourable for anti-inflammatory activity within the class of the Non-steroids. But such drugs as demonstrated in Table III tended to have serous Gastro-intestinal bleeding and infact was their drawbacks.

Gastro-intestinal bleeding and Ulceration has previously been greatly reduced by utilizing compounds which are ester analogues (6) or Cyclic analogues (7,8) of anti-inflammatory agents (NSAID). Although such analogues were not as potent as the parent compounds, they have shed light to the failure of currently used NSAIDS, as their potency was related to the intrinsic property of Gastro-intestinal ulceration.

In the design of the FK Compounds it was crucial to borrow the essential features of steroids and Non-steroidal drugs.

TABLE III

Preparation	Gastro-intestinal Bleeding GIB*	ED_{50}**	Tolerability Index GIB/ED_{50}
Diclofenac Na	17	2.1	8.1
Phenylbutazone	113	50	2.3
Indomethacin	5	5.2	1.0
Aspirin	240	900	0.3

* Dose (MG/KG) leading to blood loss of 150/MLS in 72 Hours in rats (3).

** Anti-inflammatory Potency in Rat Paw oedema Test (17,18).

A classical procedure today in the assay of anti-inflammatory activity involves the use of carrageenan to induce oedema in rat's paw. It provides information on:

(a) Whether the test substance does infact display anti-inflammatory activity

(b) It sheds light on the potency of the substance

(c) Conveys an initial impression of its Tolerability within the Pharmacologically active dose range.

The evidence that a given substance inhibits enzymes in the Arachidonic Acid Cascade maybe useful in screening of anti-inflammatory activity. But such information may not be conclusive in that Psychotropic Agents like chlorpromazine have been demonstrated to inhibit PG synthesis but do not exhibit any anti-inflammatory activity (18).

A compound combining the essential features of steroidal and Non-steroidal anti-inflammatory drugs would from a mechanistic view point possibly block the Arachidonic Acid Cascade at the two initial steps shown in Figure 2. The FK series of compounds were designed to act in such a manner and the work presented aims at demonstrating the extent at which such an approach has been achieved. It is also aimed that using standard Anti-inflammatory models to assay the FK compounds, the most potent and even superior compound to the two set of classes of anti-inflammatory drugs in use clinically, could be detected.

The experimental work was in two parts where:

(a) The pharmacological activity of the Three of the FK Series, namely, FKII - IND, FK12AC-LACT, FK13 AC-CL, were investigated against Carrageenan induced rat paw oedema. The delay of manifestation of U.V. Light induced Erythema in Guinea Pig was also utilized as a second model for the assay for Anti-inflammatory activity. The success of the latter model was limited by the fact that a Quartz U.V. Lamp was not available in order to induce an appreciable Erythema.

Indomethacin, Hydrocortisone and Dexamethasone were used as standards non-steroidal and steroidal drugs respectively in both models.

(b) The renal aspects of the three compounds were further investigated. This was crucial in that the compounds had been developed from steroidal structures. A common side-effects with steroidal anti-inflammatory drugs is their mineralocorticoid activity. The extent at which the side-effect had been eliminated was studied. Acute Toxicological tests of the Renal system was also included. A standard diuretic, Frusemide, was used as the reference for the diuresis, potassium, sodium ion excretion and for any changes pertaining to Glycosuria or proteinuria

EXPERIMENTAL WORK

The experimental work was divided into two Parts, Part A involving Experimental inflammation and Part B which was a study of the Renal aspects of the Compounds.

I Materials and Reagents:

Part A

1. Three of the FK compounds were provided (19) and included FKII - IND, FK12 AC-LACT, and FK13 AC-CL.

2. The reference drugs included Indomethacin, Hydrocortisone Sodium Succinate, and Dexamethasone. These were obtained from Kenyatta National Hospital Pharmacies, and wherever possible the drugs used were from the same manufacturer. Indomethacin was from Sigma Chemical Company, England LOT 11F-009. Hydrocortisone Sodium Succinat (SOLU-Cortef$^{(R)}$) was from Upjohn, LOT B344P and Dexamethasone was from DAWA PHARMACEUTICALS, NAIROBI, KENYA and of Control No. 59611181.

3. 1% Carrageenan in Normal Saline. Carrageenan was obtained from SIGMA CHEMICAL COMPANY, ENGLAND.

4. Male Rats of the Ratus ratus strain, from Dept. of Pharmacy Animal House were used. The preferred Range of the Rats was 150g^{+} 20g body weight.

5. A vehicle for all the drugs was 0.2% Tween 80 and 4% Polyethylene glycol in water.

PART B:

1. All the drugs and FK compounds used in A and from the same source mentioned were utilized. Frusemide from Mac's Pharmaceutics, Nairobi, Kenya was used as reference for diuretic and mineralocorticoid activity. The same vehicle was used.

2. Rat Cages were provided with every cage fitting 6 rats.

3. Flame photometer for the assay of ionic content in urine was availed.

4. Uristix Glucose and Protein strips and whatman P^H paper were used to estimate glucose, protein and P^H changes of the urine samples.

II Experimental Methods

Part A - Experimental Inflammation

Part B - Renal Aspects

A. Experimental Inflammation

(i) Carrageenan induced Rat Paw oedema (20)

All the drugs were dissolved in a vehicle containing 4% polyethlene glycol and 0.2% Tween 80 in water. If the drug was Acidic, a few drops of Dilute Sodium hydroxide were added to ensure solution. Otherwise the compounds were given as suspensions in the vehicle. Oral route by Gastric Lavage tube was used with the control animals receiving the vehicle.

One hour after drug administration, 0.1MLS of 1% Carrageenan in normal saline was injected into supplantar area of the Right Hind Paw and the initial volume (V_1) of the paw measured by mercury displacement method (Figure 3).

Exactly 3 hours later the final volume V_f of the paw was measured. For each test, 6 rats received the compound and 6 the vehicle. The swelling Δ v was calculated for each rat and hence the mean swelling for the treated rats (Δv mean treated) and that for untreated rats (Δv mean control).

The percentage inhibition of the oedema was calculated according to the following formula.

$$\% \text{ oedema inhibition} = \left\{ 1 - \frac{\Delta V \text{mean treated}}{\Delta V \text{mean control}} \right\} \times 100$$

All the male rats used in the experiment were starved 24hrs. earlier.

(ii) Delayed Manifestation of U.V. Erythema in Guinea Pig

Although the method is presented, lack of a Quartz lamp of the right intensity prevented the success of this model in the causes of the Experiment. Hence the results won't be included in the presentation.

The method of Gupta et al (21) was used. The model involves the exposure of depilated skin of Guinea-pig to U.V. Light for 20 seconds which produces prolonged inflammatory response. The erythema becomes evident within 15 to 30 minutes after the exposure and progressively increases in intensity reaching a maximum by 4 to 6hrs, and persists over 24 hrs. In the presence of Indomethacin and the FK compounds prior to exposure, the inflammatory reaction was suppressed. But the drugs were ineffective in aborting or minimizing the response when given after the inflammation is established. Corticosteroids including Dexamethasone failed to influence the U.V. inflammation in the Experiment.

Although not successful in our Laboratory, the inhibition of the Manifestation of U.V. induced Erythema is maybe the only model for testing anti-inflammatory effects giving results which could be extrapolated in man. Such a delay has been demonstrated by Grubber et al (22) and Gupta (23) in Human Skin.

B. Renal Aspects of the Compounds in Rats

Diuretic effect of the FK Compounds and any ionic, Glucose or Protein changes in urine on administration of the compounds were observed.

The cages were prepared to accomodate (Figure 4) six rats per cage. The animals had been starved 24hrs prior to the experiment.

Every Animal in the cage was given a water load of pre-warmed water of 5ML/100g body weight and placed back into the cage for 1 hour. The volume of urine flowing in a period of 1 hour was noted and sampled as the "Control" sample for that drug or vehicle.

The animals were then fed by gastric lavage with the drugs prepared in the vehicle 0.2% Tween 80 and 4% polyethylene glycol, given as a solution or suspension of 1ML/100g body weight. The dosages had been standardised to 4MG/KG body weight, but for Hydrocortisone a further Dose of 100MG/KG was used.

These were the doses with an appreciable (ED_{50}) response in the inhibition of the paw oedema (A).

Further samples of urine were taken every 30 minutes for a period of 24hrs. This led to 30, 60, 90, 120, 150, 240 minutes and 24hrs samples. The PH, glucose and protein content were tested using a whatman PH paper indicator and uristix(R) glucose and protein strips respectively. The tests were done immediately whenever possible but not later than 12Hrs. after the last sample of urine.

Urine samples were diluted by factor of 100 or even 1,000 and against standard potassium chloride and sodium chloride (Na^+ and K^+ ions) tested for Na^+ and K^+ ions using flame photometry. (Figure 5).

All deaths occuring in the 24Hrs of the Experiment were recorded and if possible investigated to rule out possibility of the drug to be the cause of the death.

In a similar group of six rats the vehicle was given as a solution of 1ML/100g body weight of rat. Similarly, Frusemide was given as a control for diuresis and "acceptable" ionic excretion or glucose and protein changes.

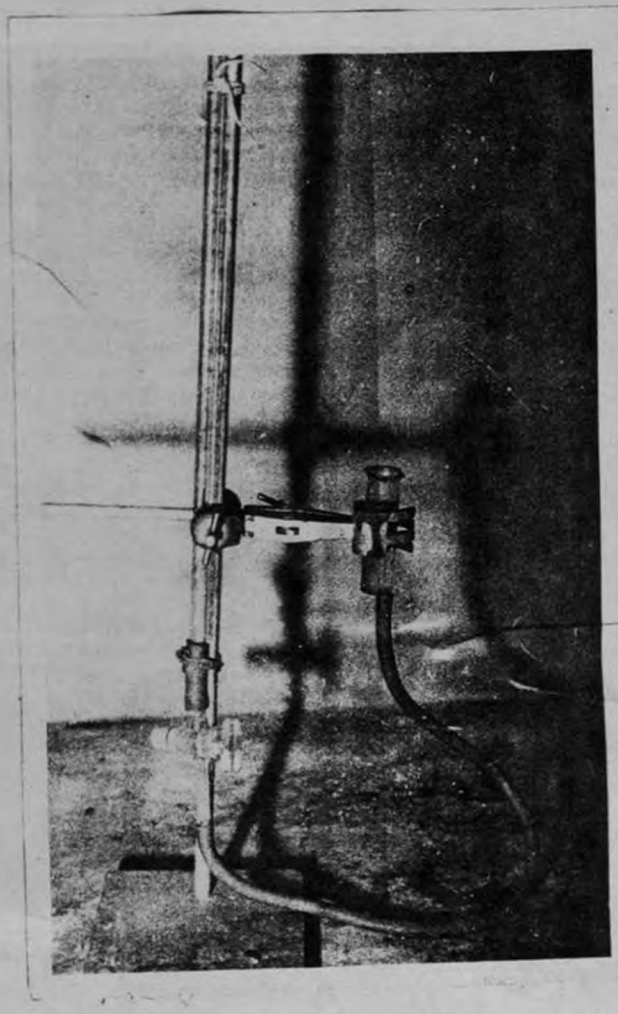


Fig 3 (i)

Typical apparatus used to measure the volume of the Paws of rats.

The level of Mercury displaced on insertion of the paw is read on the Burette.

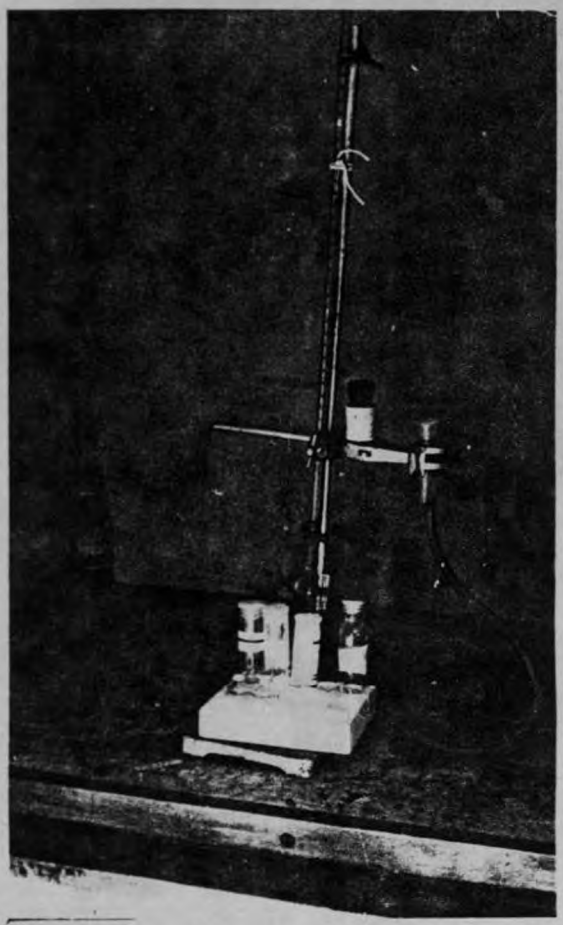


Fig 3 (ii)

Typical apparatus used to measure the volume of the Paws of rats.

The level of Mercury displaced on insertion of the Paw is read on the Burette.

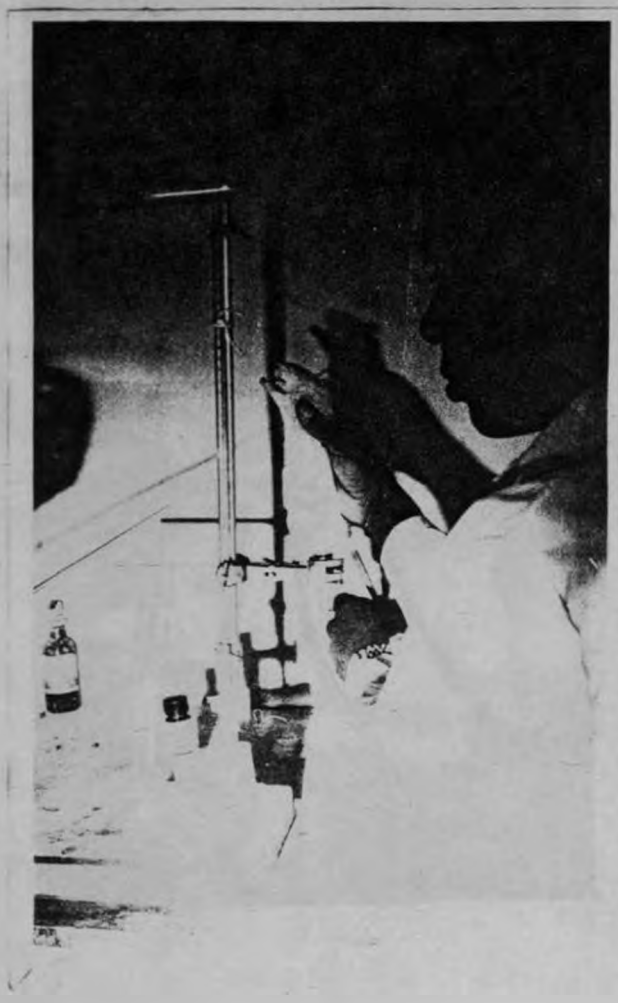


Fig 3 (iii)

The Author is seen measuring the volume of the left Paw.

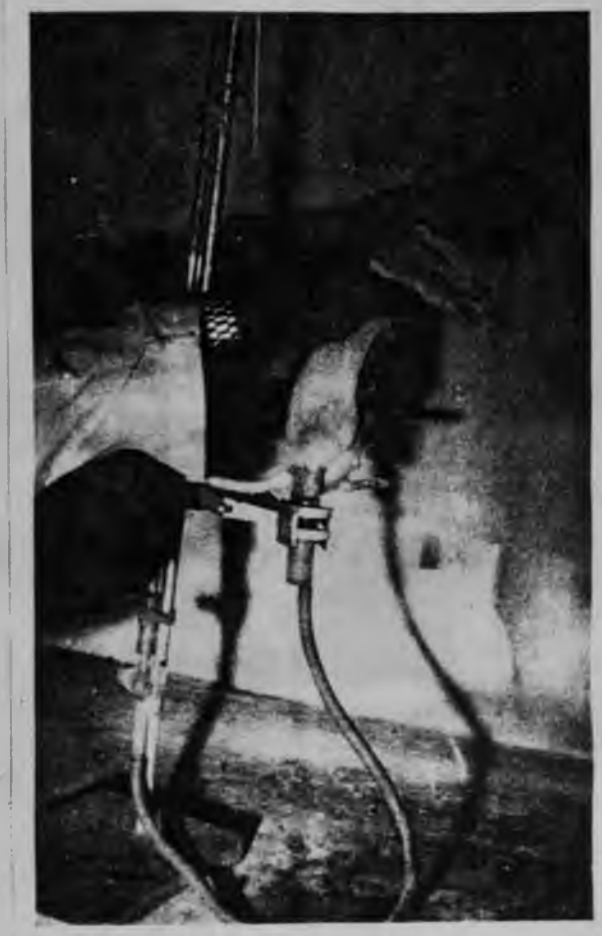


Fig 3 (iv)

The Author is seen measuring the volume of the Right Paw, after injection with carrageenan.

Fig 3 (v)

The photograph shows a contrast between the Right Paw (arrow) and the Left Paw.

The Right Paw has been "treated" with carrageenan whereas the Left Paw was not.

Fig 3 (vi)

The two hind paws are of comparable volumes due to inhibition of the carrageenan induced oedema in a rat pre-treated with FK13AC-CL.

The Right paw was "treated" with carrageenan 1 hour after the oral administration with the drug.

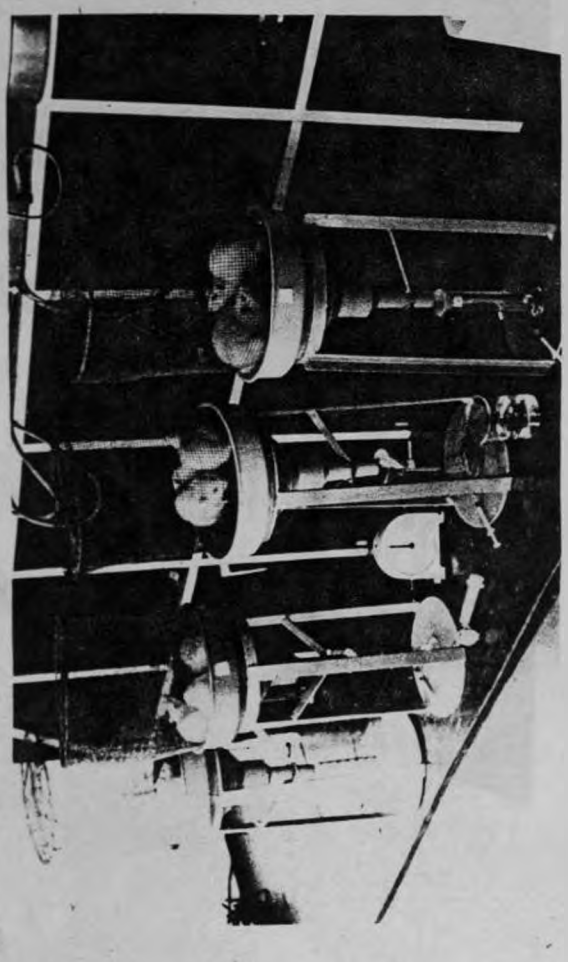


Fig 4

- Typical Rat Cages
  Used to accomodate six rats (per cage) during the study of the Renal Aspects.

- Urine was collected for 24 hours and Biochemical changes studied as explained in the text.

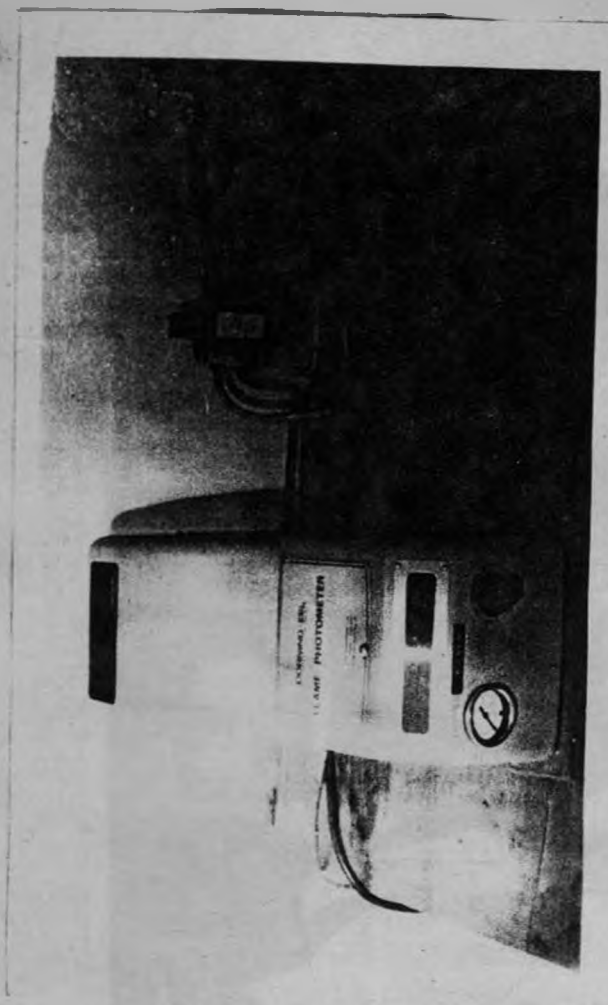


Fig 5

Flame photometer used in the Assay of Ionic content (Potassium and Sodium) in the Urine samples.

Fig 5 (b)

Macroscopical Examination of the Gastrointestinal tract (Git) for any Bleeding or Ulceration. 15 minutes after treatment with Anti-inflammatory Agents.

R E S U L T S

A Inhibition of Carrageenan induced Rat Paw Oedema

Figure (6) is the plot of % inhibition of Paw Oedema against the Log Dose. Every point in the plot was the mean response for six Animals. The FK compounds were parallel showing that their mode of Action was the same.

At low doses FK13 AC-CL was more potent than indomethacin. From the plot the anti-inflammatory activity of the compounds could be summarised as in Table IV.

Anti-inflammatory Activity:- Table IV

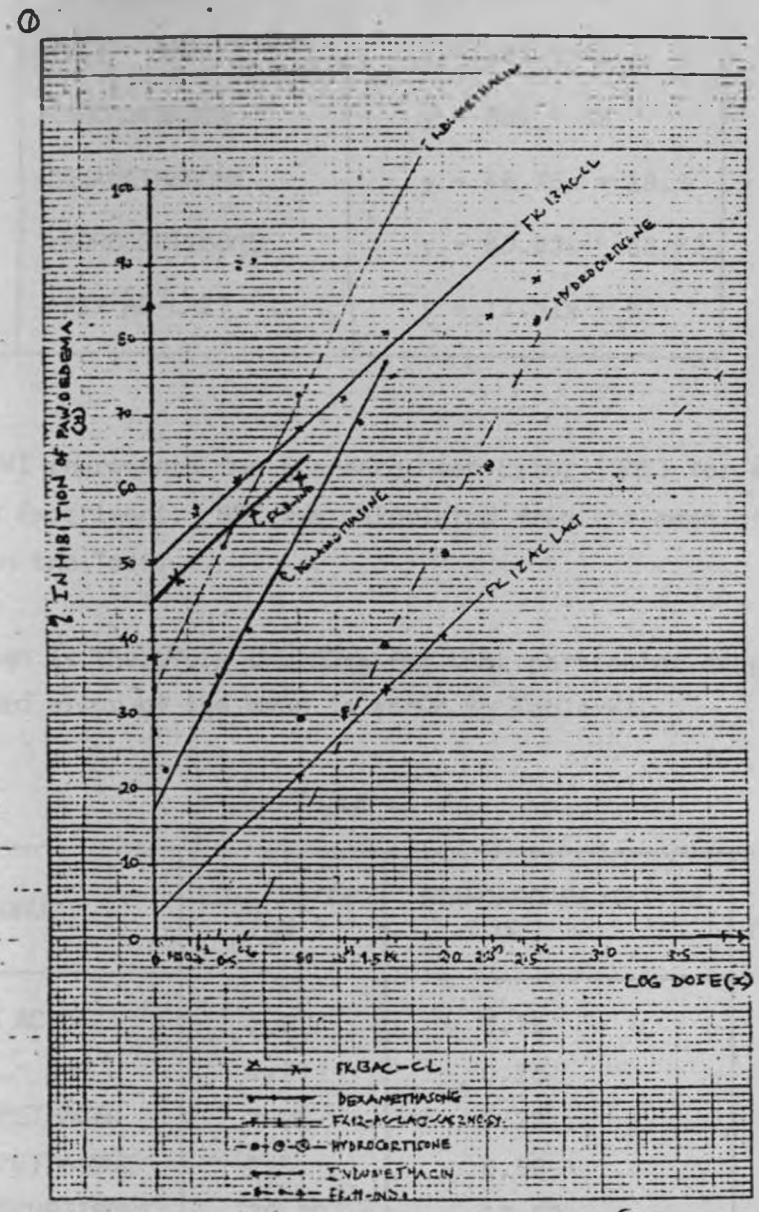
NB: ED_{50} = Dose of drug required to inhibit paw oedema by 50% maximum possible inhibition. Likewise for ED_{75}.

DRUG	ED_{50}	ED_{75}
FK13 AC-CL	1.0 Mg/Kg	25Mg/Kg
FK11 - IND	1.78 Mg/Kg	44.6Mg/Kg
INDOMETHACIN	2.82 Mg/Kg	10.17Mg/Kg
DEXAMETHASONE	7.5 Mg/Kg	35.48Mg/Kg
HYDROCORTISONE	70.79 Mg/Kg	298.54Mg/Kg
FK12 AC-LACT	316 Mg/Kg	6683Mg/Kg
ASPIRIN*	900 Mg/Kg	-

* Krupp et al (17,18).

The Equation describing the plot of percentage inhibition of the paw oedema or the anti-inflammatory response (y) to the logarithm of the Dosage (x) are represented in Table V.

FIG. 6



Plot of % inhibition of paw oedema with time using male rats of 150g⁺± 20g

- All drugs were given in a vehicle of 0.2% Tween 80 and 4% polyethylene Glycol in water.
- The doses chosen were 1, 2, 3, 4; 10, 20, 40 and 100, 200, 400 Mg/Kg for every compound (drug) in the plot.
- Every point in the graph is a mean of 6 rats.

Table V

DRUG	EQUATION
FK13 AC-CL	y = 18x + 46
FK11 - IND	y = 19x + 45
INDOMETHACIN	y = 38x + 33
DEXAMETHASONE	y = 46.75x + 18.5
HYDROCORTISONE	y = 63.63x - 82.72
FK12 AC-LACT	y = 17.31x + 5

Table VI represents the Standard Mean Error (SME) for every ED_{50} used in the Experiment. SME was calculated from the mean of the six animals used in the Test.

The Mean is therefore the ED_{50} for that particular drug, and the standard error of the mean is shown in Table VI.

TABLE VI

DRUG	ED_{50} Mg/Kg (μ)	STANDARD DEVIATION (σ)	STANDARD MEAN ERROR ($\sigma/\sqrt{n}$)
FK13 AC-CL	1.0	0.15	0.06
FK11 - IND	1.78	0.18	0.07
INDOMETHACIN	2.82	0.22	0.089
DEXAMETHASONE	7.5	0.59	0.24
HYDROCORTISONE	70.79	13.69	5.58
FK12 AC-LACT	316	21.81	8.9

The Parameters in the Table were calculated using the following method:

FK13 AC-CL

ANIMAL	DOSE CAUSING 50% RESPONSE (Mg/Kg) (x_i)
1	1.0
2	1.06
3	0.9
4	0.8
5	0.8
6	1.2

Mean dose μ = 1.0 and n = 6

Standard Deviation σ

$$\sigma = \sqrt{\left\{\frac{\sum(x_i-\mu)^2}{n}\right\}} = 0.15$$

Standard Mean Error (SME)

$$SME = \sigma/\sqrt{n} = 0.06$$

B. RENAL ASPECTS

In this set of experiments the Number of dying animals was recorded. All the deaths occurred between 16 and 24 hrs after drug administration, Table VII

TABLE VII

DRUG	NUMBER OF DEATHS (16 to 24 Hrs)	NUMBER OF ANIMALS PER CAGE
DEXAMETHASONE	0	6
INDOMETHACIN	1	6
FK13 AC-CL	2	6
FK12 AC-LACT	2	6
FK11 - IND	3	6
VEHICLE	2	6
FRUSEMIDE	1	6

There was a close correlation between the number of dying Animals and those found to have been eaten by others. Hence it was concluded that the deaths could not have been as a result of the Drugs but due to Hunger and crowding effect, expected in the cages used (Figure 4).

The biochemical changes of urine samples collected over a period of 24 hrs are shown in CHART NO. 2. The mean urine PH, glucose and protein content was taken in urine samples after administration of the drugs.

No glucose was detected in the urine in all cases. There was a general increase in the protein content in all the cages including the rats fed on the vehicle. Any changes in protein content of urine could therefore not have been a drug effect but to some pathophysiological changes within the rats not related to the experiment.

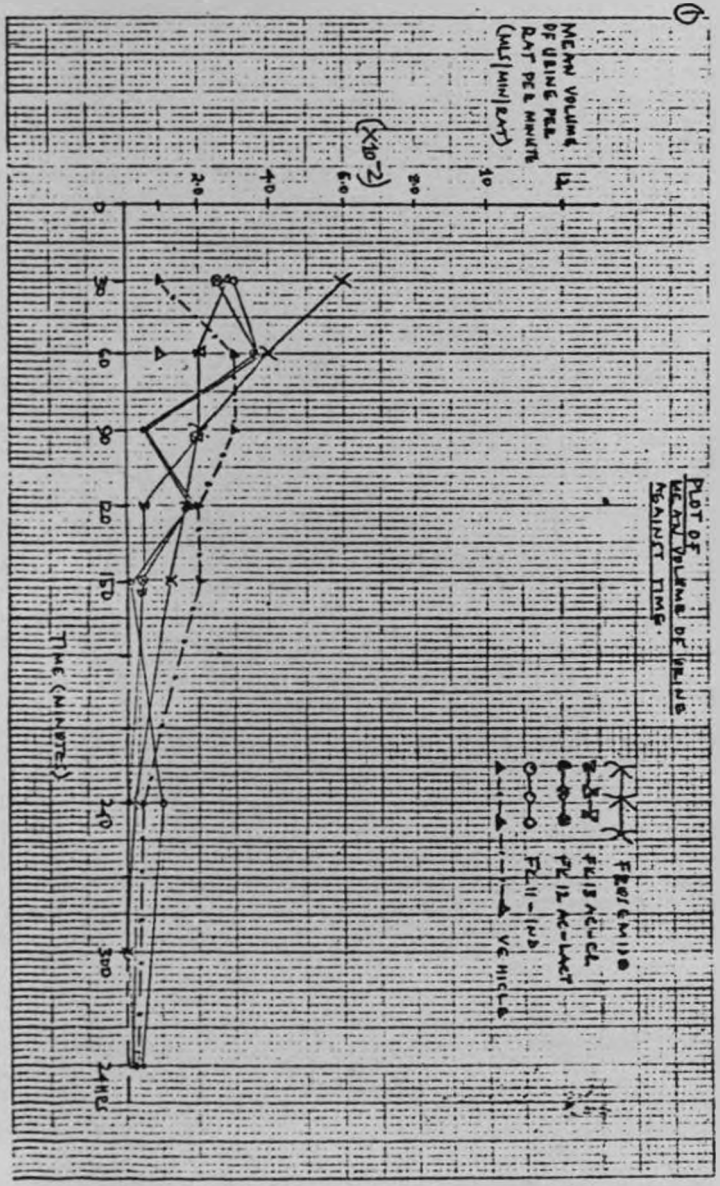
The FK compounds didn't show any diuretic effect compared to Frusemide. The mean volume of urine with time in charts (1 and 3) are shown in FIGURE 7. The diuretic effect of the FK compounds was therefore comparab with the vehicle.

The Mineralocorticoid Activity of the FK compounds was studied. The level of sodium and potassium in urine for all the compounds determined by Flame photometry for a period of 24 hrs after administration of the drugs are shown in CHART NO. 5. The mineralocorticoid activity was further enumerated in the Plots of sodium ion in urine against time (Fig. 8) and potassium ion in urine against time (Fig. 9).

In Figure (10) the potassium ion in urine for the FK compound was compare to that shown by the vehicle.

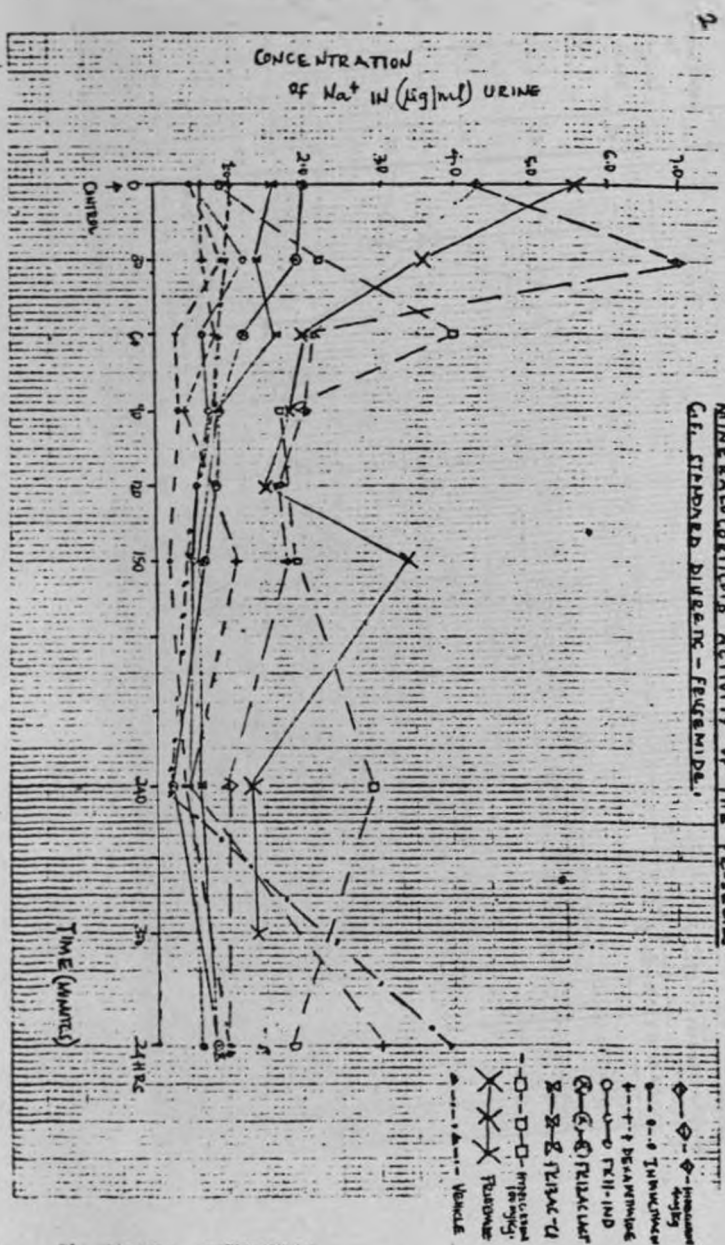
Finally, the cummulative plots for potassium in urine versus cummulative volume of urine was described for FK13 AC-CL, FK12 AC-LACT, and FK11 - IN in Figure 11. The plot shows that the ionic changes and diuretic activit were superimposed. FK13 AC-CL was further compared with Indomethacin and the vehicle in Figure 12.

FIG. 7



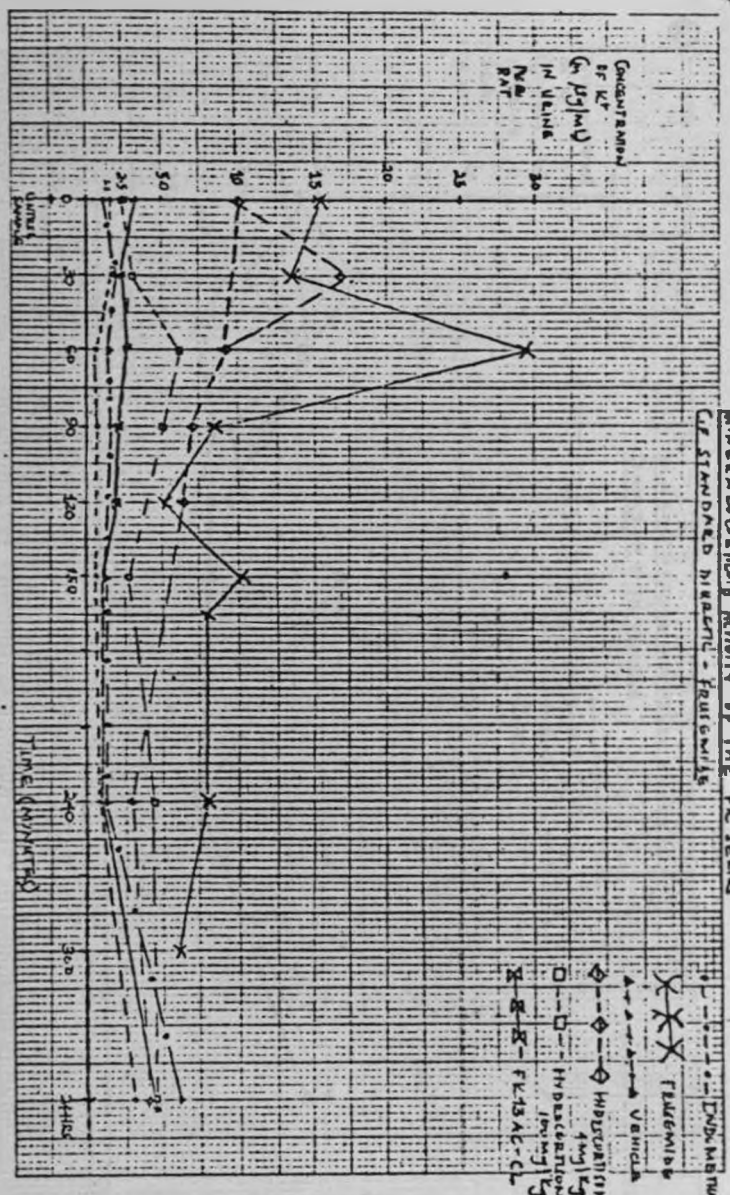
Explanation in text

FIG. 8



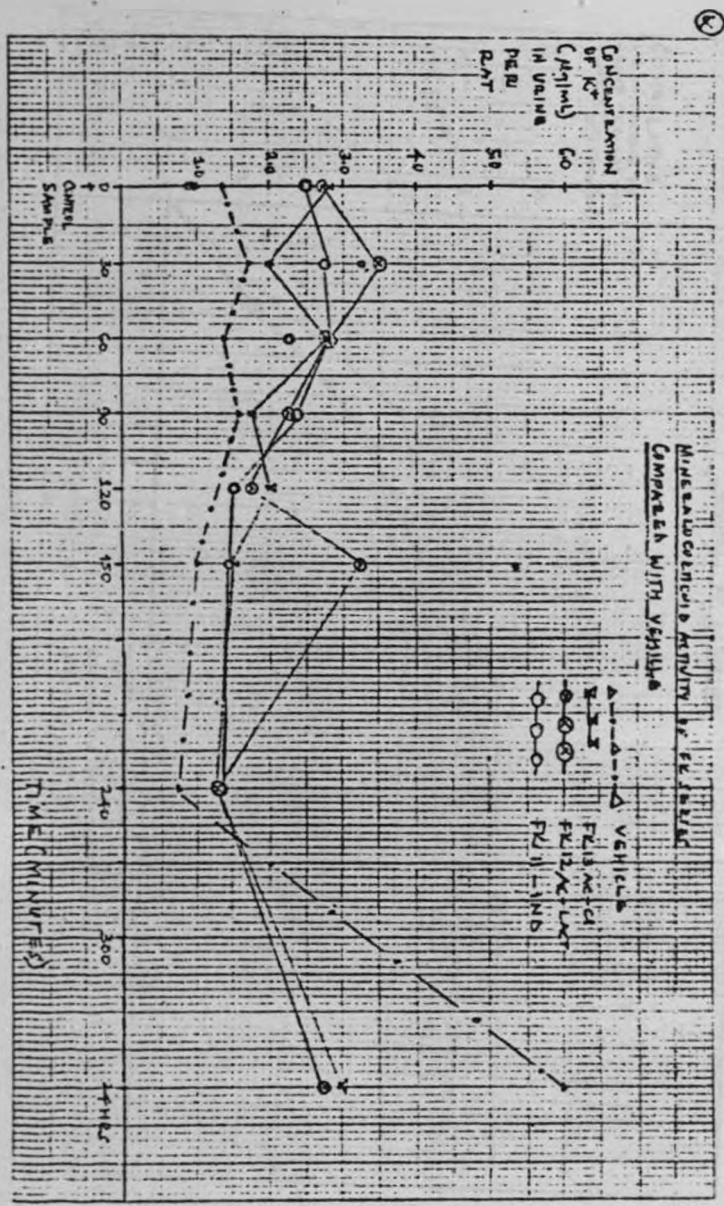
Explanation in text

FIG. 9



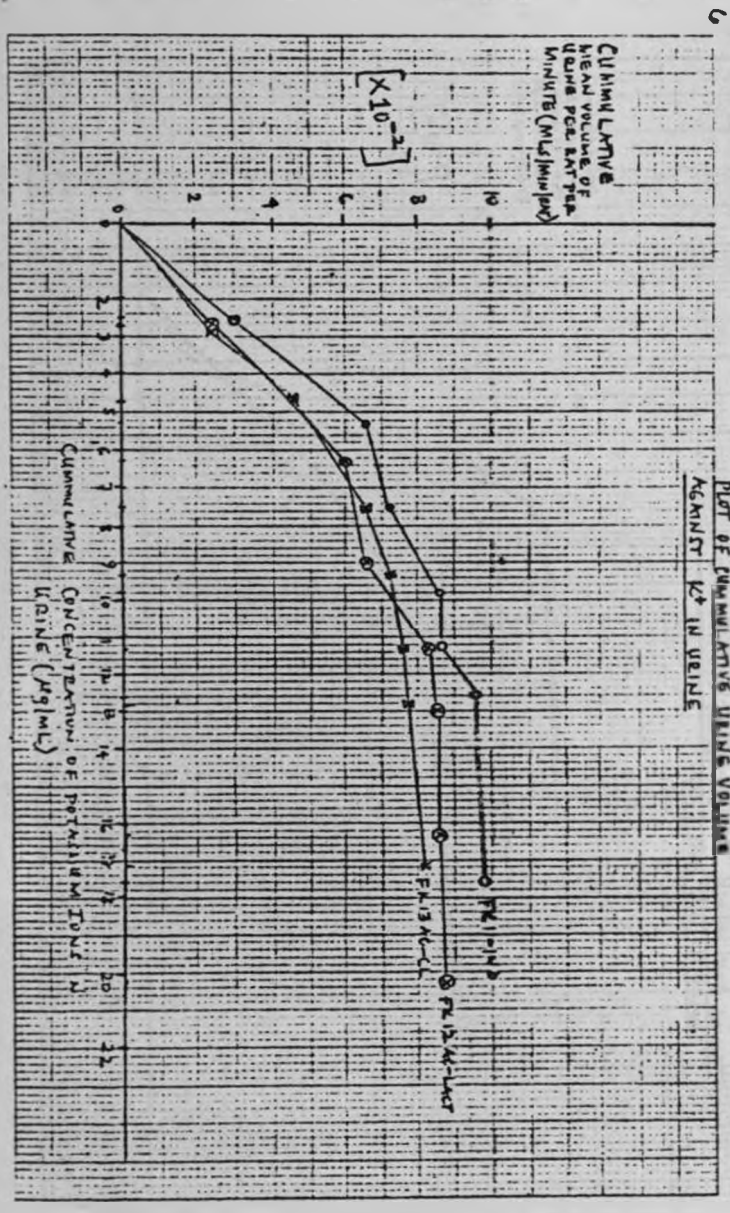
Explanation in text

FIG. 10



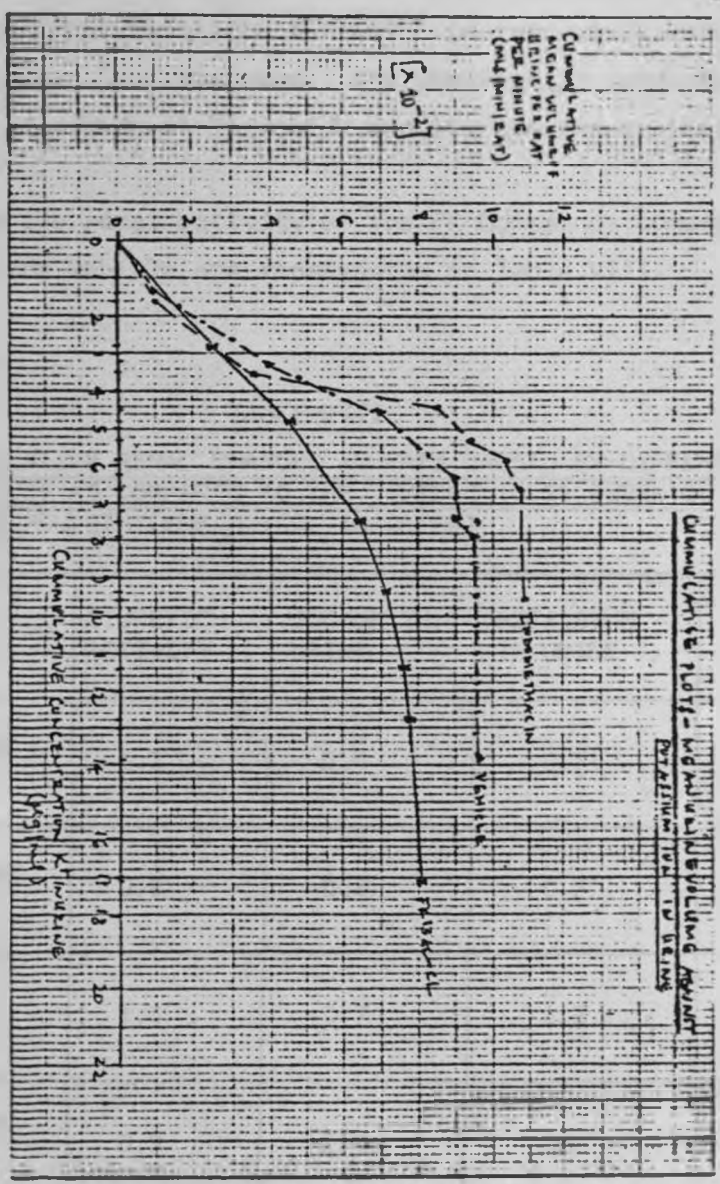
Explanation in text

FIG. 11 .



Explanation in text

FIG. 12



Explanation in text

The latter plot (Figure 12) shows that while Indomethacin had a greater diuretic activity than the vehicle and FK13 AC-CL respectively, FK13 AC-CL had a total excretion of potassium ion greater than that of the vehicle and Indomethacin respectively.

But in all the drugs experimented changes in potassium, sodium or diuretic activity were minimal compared with Frusemide. Infact it was demonstrated that potassium excretion in rats was greatest with Frusemide than any other compound. The observation tends to agree with the mode of action of Frusemide and its use today clinically.

The summary of the renal activity of the FK Compounds compared to Frusemide and the vehicle are represented in Table VIII.

TABLE VIII

RELATIVE RENAL CHANGES IN RATS CAUSED BY DIFFERENT DRUGS AS COMPARED TO THE VEHICLE (0.2% TWEEN 80 and 4% POLYETHYLENE GLYCOL IN WATER)

DRUG	DIURETIC ACTIVITY	SODIUM IN URINE	POTASSIUM IN URINE	PH
INDOMETHACIN 4Mg/Kg	1.1	0.4	0.7	7.0
DEXAMETHASONE 4Mg/Kg	0.9	0.8	0.7	7.8
FKII-IND 4Mg/Kg	1.0	0.7	1.2	10
FK12 AC-LACT 4Mg/Kg	0.9	1.0	1.5	8.0
FK13 AC-CL 4Mg/Kg	0.8	1.0	1.2	9.6
HYDROCORTISONE 4Mg/Kg	0.8	2.5	4.0	9.0
HYDROCORTISONE 4Mg/Kg	1.0	2.1	2.0	9.5
FRUSEMIDE 4Mg/Kg	2.5	3.0	7.0	9.75
VEHICLE 10ML/Kg	1.0	1.0	1.0	7.2

DISCUSSION

The results on Experimental inflamation in Table IV and Table VI demonstrates that two of the Novel series of Compounds, FKII-IND and FK13 AC-CL were more potent that Indomethacin in the inhibition of Carrageenan induced paw oedema.

FK12 AC-LACT was developed to act as a pro-drug and that possibility has been demonstrated in the Delayed castor oil induced colonic water flux Model (25). A higher dose was therefore required for FK12 AC-LACT in the Carrageenan Model which causes an inflammation of an Acute nature. The chronic nature of the colonic water flux allows the conversion of the FK12 AC-LACT to its Active forms and hence the greater activity observed.

The fact that the plot of percentage Inhibition of paw oedema to LOG Dose (Fig.6), the FK Compounds are parallel, describes possible similar mode of Actions. At low doses ($< ED_{60}$) FK13 AC-CL maybe acting similarly to Indomethacin whereas at higher doses ($> ED_{75}$) the Compound is Proposed to mimic the steroidal drugs.

Infact, Gupta (23) has demonstrated that Corticosteroids fails to cause a delay in inflammation in pre-treated guinea-pigs when exposed to ultra violet light. It suffices that for a compound with steroidal and Non-steroidal mechanistic of action in bringing about anti-inflammatory activity, will cause a Delay in Manifestation of U.V. Erythema. Such a possiblity has been demonstrated but using a weak U.V. Lamp. Better results are to be expected on a Quartz Lamp, Hanovia Analytical Model.

The usefulness of prodrugs like FK12 AC-LACT cannot be disputed today especially as they act as slow-reacting anti-rheumatic Agents. It is in such a manner that chloroquine finds use in rheumatoid arthritis. The same is true for D-penicillamine and Levamisole, which infact have been suggested to suppress immunological mediators of inflammation (26). The latter two drugs fail to inhibit prostaglandins release in vitro when the concentration used were in the same range as the human blood levels observed during Therapeutic doses. Contrary Indomethacin produces significant inhibition on in vivo and in vitro tests (26).

The Evaluation of toxicity studies in anti-inflammatory agents have mainly been centred at Gastro-intestinal bleeding and ulceration. The changes are normally assessed on the basis of macroscopical and microscopical examination of the stomach and intestine after sacrificing pre-treated animals with doses well above the oral LD_{50}.

Renal toxicity caused by anti-inflammatory agents is today of equal importance due to reports linking salicylate therapy, Gold, Steroids and phenylbutazone usage to renal changes (27, 28, 29, 30). Acute Renal toxicity testing involving the investigation of urine could be combined with studies of Gastro-intestinal ulcerogenic effects. A recent study has utilized urine analysis (recovery) of phenol red (phenolsulfonphthalein) in rats pre-treated with the drug and 15 hours later phenol red (31). The absorption of phenol red after oral administration is minimal. But in Gastro-intestinal ulceration it is absorbed in appreciable levels. Any absorbed phenol red undergoes minimal metabolism and Renal Tubular secretion is pronounced such that most of the absorbed phenol red appears in urine in 4hours. The increase in levels of the marker recovered in urine in Drug-Pretreated rats as opposed to the untreated rats is used as an ulcerogenic index.

Ulcerogenic effects caused by FK Compounds compared to steroidal and non-steroidal anti-inflammatory drugs is being investigated utilizing the phenol red urine recovery method.

From Table VIII the following conclusions were made on the evaluation of the Renal toxicity of the FK compounds:-

- FKII-IND, FK12 AC-LACT and FK13 AC-CL had no net diuretic effect compared with the vehicle of 0.2% Tween 80 and 4% Polyethylene Glycol in water
- The ionic content of urine, mainly, potassium and sodium was comparable to that produced on administration of the vehicle.
- Frusemide as expected had a net excretion of potassium ions
- The FK compounds were comparable to indomethacin as far as diuretic and ionic content of urine was concerned.

. The FK compounds led to a negative result in urine glucose, whereas protein was always present in urine irrespective of whether the drugs or vehicle were administered. Hence protein in urine was not a drug effect but due to some unexplained pathophysiological phenomena in the rats.

During the pharmacological screening for Anti-inflammtory activity and in the renal toxicity evaluation of these compounds, the oral route of administration was preferred. This is in agreement with the observation that the oral route is most favourable in man. Steroids are not normally administered via this route due to the "Fast Pass effect". Since the FK compounds were shown to be potent in the route, their steroidal characteristic as far as the "Fast Pass effect" is concerned may have been eliminated. At the same token the need for the screening for anti-inflammatory effect using other routes of administration, parenteral and Topical, cannot be under looked.

Without any Renal changes being detected in the acute usage of the FK compounds was suggestive that a certain safety Margin exists which could be exploited. Formulation studies of these compounds would need be intiated. In such studies and other pre-clinical trials in Animals and Humans, the potential for Gastro-intestinal toxicity and chronic renal changes needs be defined. The Biopharmaceutical and Pharmacokinetic parameters of these compounds would give the clinician an idea on the absorption, distribution, metabolism and Elimination. Distribution in certain organs over the others is usually an advantage in most therapeutic usages of drugs, and tends to define the efficacy and possible mechanisms of Toxicity of the drug (24). It is an aspect applied within the anti-inflammatory drugs.

Findings that an anti-inflammatory Agent has a high uterus to plasma ratios than other similar drugs has led to the observation that such a drug maybe useful in Treatment or Management of the Pain in Dysmenorrhoea(32). The therapeutic interest in dysmenorrhoea gained a new momentum with the recognisation of the possible role of uterine prostaglandins in the pathogenesis of the condition (33). With the discovery that anti-inflammatory agents inhibit prostaglandin synthesis so was the rationale in the use of Indomethacin, Flufenamic Acid, Naproxen in the condition. The use of salicylates in the condition is limited by the observation that the group has anti-platelet activity and would therefore lead to the prolongation of uterine bleeding.

$PGF_{2\alpha}$ has been recovered in menstrual fluid in appreciable levels in dysmenorrhoeic patients and a causal association suggested. It has also been indicated to cause other symptoms commonly observed in dysmenorrhoeic patients especially involving the Gastro-intestinal symptoms of vomiting, crumps and diarrhoea (34).

The usefulness of the FK compounds in Topical application would need to be investigated. The delay in the manifestation of UV erythema model has been used and provides a means for the assessment of Topically applied anti-inflammtory drugs in Humans (22). The model has been used in the study of the vehicle with the best release properties of the drug. Again with the future of Transdermal medication developing rapidly, use of NSAID in conditions like arthritis may utilize the route (35). The best results are obtained with drugs of high lipid and water solubitity and where partition coefficient is between 1 and 2. Patients compliance would be higher because of the ease of administration of the drug as a cream or ointment.

Special Tests Like Teratogenicity and carcinogenecity would need to be initiated on these compounds. The use of more than two species in such tests a rodent and non-rodent is recommended by the World Health Organisation (WHO) (36, 37). Pharmacogenetic tests are also useful and need be evaluated. But like Toxicity related to Central Nervous System (CNS), like headache, psychosis and peripheral neuritis; pharmacogenetic tests cannot be assessed in animals and Human Tests during clinical trials or clinical usage of the drugs in humans would need be performed.

The final assessment of a new anti-inflammatory agent would depend on many years evaluation in arthritic patients. The same is true on adverse reactions resulting in drug usage especially if the reactions are of immunological nature. Immunological Tests are not conclusive and would always demand that the drug be exposed to the particular individual concerned.

In any case, provacative tests *in vivo* are dangerous even when small doses of a drug are used as an anaphylactic attack may occur. Cross-reactivity have been observed not only with related group of drugs but with food and drug adjuvants. The phenomena has been observed with the dye tartrazine which was found to cross-react with salicylates in Asthmatics (38).

Infact the use of the dye to colour formulations of antibiotics and bronchodilators for asthmatics need be restricted if not contra-indicated.

From the work presented, a novel series of compounds bridging steroidal and non-steroidal features of anti-inflammatory agents have been synthesized. FKII-IND and FK13 AC-CL were more potent than Indomethacin whereas FK12 AC-LACT though not as active is proposed to be a pro-drug with obvious advantages. Extensive studies would need be performed to ascertain the extent at which advantages observed with steroidal and non-steroidal anti-inflammatory drugs, imparted in the representative FK compounds tested, have been compromised by the elimination of undersirable effects seen within the two groups of drugs. Further work related to structural activity relationship of this novel series of compounds is also called for. This is especially true for FK12 AC-LACT where the study of metabolites may allow confirmation of the possibility that it acts as a pro-drug.

Otherwise, the FK compounds presents a novel and ideal anti-inflammatory agents which need be assessed with their ultimate use in clinical medicine.

VOLUME OF URINE PER RAT PER MINUTE (MLS/MIN/RAT)

TIME	0 TO 30MIN	30 TO 60MIN	60 TO 90 MIN	90 TO 120 MIN	120 TO 150 MIN	150 TO 240 MIN	240 MIN TO 300 MIN OR 24 HRS
INDOMETHACIN 4Mg/Kg	0.01	0.027	0.047	0.01	0.01	0.004	0.0018
DEXAMETHASONE 4Mg/Kg	0.005	0.02	0.033	0.01	0.01	0.005	0.005
FKII - 1ND 4Mg/Kg	0.030	0.036	0.005	0.018	0.018	0.018	0.0024
FK 12 AC LACT 4Mg/Kg	0.025	0.036	0.005	0.018	0.003	0.003	0.0024
FK 13 AC - CL 4Mg/Kg	0.025	0.02	0.02	0.005	0.005	0.001	0.004
HYDROCORTISONE 4Mg/Kg	0.01	0.008	0.02	0.03	0.003	0.003	0.005
HYDROCORTISONE 100Mg/Kg	0.025	0.005	0.03	0.03	0.03	0.008	0.005
FRUSEMIDE 4Mg/Kg	0.08	0.04	0.02	0.017	0.012	0.0038	0.005
VEHICLE	0.01	0.03	0.02	0.02	0.004	0.004	0.003

CHART NO: 1

"DIURETICS"

1. NO OF RATS PER DRUG OR VEHICLE WERE SIX PER CAGE. EACH DRUG WAS GIVEN ORALLY AS A VOLUME OF 1ML/100g BODY WEIGHT RAT IN A VEHICLE OF 0.2% TWEEN 80 IN 4% POLYETHYLENE GLYCOL.
2. THE ANIMALS WERE FASTED 24HRS PRIOR TO EXPERIMENT AND WERE GIVEN A WATER LOAD OF 5ML/100g BODY WEIGHT PRIOR TO DOSING.

BIOCHEMICAL CHANGES AFTER DRUG ADMINISTRATION

DRUG	MEAN URINE PH IN 24 HRS	GLUCOSE IN URINE (24 HRS)	PROTEIN IN URINE (24 HRS)
INDOMETHACIN 4Mg/Kg	7.0	0	++
DEXAMETHASONE 4Mg/Kg	7.8	0	++
FKII 1 INO 4Mg/Kg	10.0	0	+++
FK12 AC LACT 4Mg/Kg	8.0	0	++
FK13 AC - CL 4Mg/Kg	9.6	0	+++
HYDROCORTISONE 4Mg/Kg	9.0	0	++
HYDROCORTISONE 100Mg/Kg	9.5	0	++
FRUSEMIDE 4Mg/Kg	9.75	0	+++
VEHICLE	7.2	0	++

CHART NO: 2

"DIURETICS"

PROTEIN:- + = 30Mg/100ML
++ = 100Mg/100ML
+++ = 300Mg/100ML
++++ = 1,000Mg/100ML

GLUCOSE:- 0 = NEGATIVE
+ = LIGHT
++ = MEDIUM
+++ = DARK

URINE SAMPLES WERE COLLECTED PER CAGE FOR 24 HRS WHERE EACH CAGE HAD SIX RATS.

CUMMULATIVE MEAN VOLUME OF URINE PER RAT PER MINUTE (MLS/MIN/RAT)

TIME	30 MIN	60 MIN	90 MIN	120 MIN	150 MIN	240 MIN	300 MIN	24 HRS
INDOMETHACIN 4Mg/Kg	0.01	0.037	0.064	0.084	0.104	0.108	-	0.1086
DEXAMETHASONE 4Mg/Kg	0.005	0.025	0.058	0.068	0.078	0.083	-	0.088
FK-11-1ND 4Mg/Kg	0.03	0.066	0.071	0.087	0.087	0.097	-	0.0995
FK 12 AC LACT 4Mg/Kg	0.025	0.061	0.066	0.082	0.085	0.085	-	0.0874
FK 13 AC-CL 4Mg/Kg	0.025	0.045	0.065	0.071	0.076	0.077	-	0.081
HYDROCORTISONE 4Mg/Kg	0.01	0.018	0.038	0.068	0.071	0.074	-	0.079
HYDROCORTISONE 100Mg/Kg	0.025	0.030	0.06	0.09	0.09	0.098	-	0.101
FUSEMIDE 4Mg/Kg	0.06	0.1	0.12	0.137	0.149	0.1526	0.1526	0.1526
VEHICLE	0.01	0.04	0.07	0.09	0.09	0.094	-	0.097

CHART NO: 3

"DIURETICS"

READINGS FOR THE STANDARD CURVE

CONCENTRATION Na^+ MICROGRAM PER MILLILITRE	AVERAGE READINGS	CONCENTRATION K^+ MICROGRAM PER MILLILITRE	AVERAGE READINGS
1	8	2.5	16
2	19	5	27
4	34.5	10	49
8	58.5	15	71
10	72	20	87
15	100	25	100

CHART NO: 4

TIME	CONTROL		30 MIN		60 MIN		90 MIN		120 MIN		150 MIN		240 MIN		300 MIN OR 24 HRS	
ION	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+
INDOMETHACIN 4Mg/Kg	0.5	1.75	0.8	1.80	0.3	0.75	0.32	0.95	-	-	0.2	0.6	0.4	0.75	1.0	3.0
DEXAMETHASONE 4Mg/Kg	0.6	0.75	0.81	1.0	0.8	1.25	0.4	0.55	-	-	1.1	1.0	0.49	0.95	3.0	4.5
FK11 - IND 4Mg/Kg	0.95	2.4	1.2	2.75	0.81	2.25	0.7	2.30	0.55	1.5	0.5	1.45	0.49	1.30	0.81	2.75
FK 12 AC LACT 4Mg/Kg	2.0	2.75	1.8	3.5	1.2	2.75	0.85	2.25	0.80	1.75	0.85	3.25	0.24	1.25	0.85	2.75
FK 13 AC-CL 4Mg/Kg	1.8	2.8	1.4	2.0	1.85	2.75	0.89	1.75	0.85	2.0	0.80	1.5	0.60	1.3	1.1	3.0
HYDROCORTISONE 4Mg/Kg	4.3	10.0	7.0	16.25	2.1	9.90	2.0	7.0	1.7	6.45	1.8	0.75	1.0	2.8	0.9	3.0
HYDROCORTISONE 100Mg/Kg	0.9	2.5	2.2	3.25	4.0	8.0	1.7	2.25	1.65	2.15	1.9	2.75	3.4	4.0	1.9	3.5
FRUSEMIDE 4Mg/Kg	5.6	15.5	3.6	13.5	2.0	29.5	1.8	8.5	1.5	5.05	3.4	10.05	1.8	8.05	1.9	6.75
VEHICLE	1.0	1.4	0.9	1.75	0.85	1.4	0.80	1.6	-	-	0.5	1.0	0.3	0.75	3.9	6.0

CHART NO: 5 NB: (1) $[Na^+]$ and $[K^+]$ are for a single Rat Taken from the Readings of Six Rats Per Drug.

UNITS = μg/ML.

(2) The Mean Weight Per Rat of the Group was 200g $\pm$ 10g

(3) Male and Female Rats were used from a random selection from Animal House.

(4) Flame photometry was used to Assay the ionic content in urine.

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