TOWARDS THE BIOASSAY OF A NOVEL SERIES

OF

SYNTHETIC - PUTATIVE FK ANTI-INFLAMMATORY AGENTS

BY

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THEY WERE TOTALLY COMMITTED, THEY SUFFERED,
THEY BLED, THEY WERE SACRIFICED:

- When I returned to the forest, I found comrades safe and well,
  As comrades-in-arms we embraced one another with joy,
  We are committed to the liberation of Kenyan people,
  Praise be to Mwene - Nyagah

  Chorus: Follow behind the youth
  And remember
  This country is forever ours.

  (Maina wa Kinyatti - "Mau Mau Patriotic Songs)

- I have fed out of drum, I have drunk out of cymbal,
  I have entered your bridal chamber ("Distances"),
  So would I to the Hills again, so would I,
  To where springs the fountain, there to draw from,
  And to the hill top, body and soul,
  White washed in moondew, there to see from ("Heaven's gate").

  (by Christopher Okigbo)

- "Why do you catch hell? Why do all of us catch hell?
  Not because we are Muslim nor Christians, Catholic nor Protestant,
  Baptish nor Methodist, Mason nor Elk,
  You catch hell; all of us catch hell since we all are black men
  'cos if we are white men we would surely catch no hell!

  (EL - Hajj Malik, EL - Shabaaz (MALCOM X)

- I have a dream to-day my friends .... even though we face
  difficulties to-day and tomorrow ...., I still have a dream
  that one day man will not be judged by colour of their skin
  but by content of their character ....
  I have a dream that the brotherhood of men will become a
  reality .... I HAVE A DREAM! That ....
  - only when its dark enough can you see the stars -

  (Dr. Martin Luther King Jr.)

- AND FOR THE ALBINO RATS THAT DID SUFFER, THAT BLED, THAT UNDERWENT SACRIFICE, IT WAS TOTAL COMMITMENT, WHAT A CONTRIBUTION TOWARDS FK COMPOUNDS! WHAT A LONG LASTING CONTRIBUTION TO SCIENCE!

(author)

DEDICATIONS

. Ndirangu wa Kahato	-	My dear father, whose love for education is unparalleled. You command my utmost admiration.
. Waithira wa Ndirangu	-	Dear mother, your contribution to all that I am is colossal. Always in communion.
. Kahato wa Kiunge (Guka)	-	The most revered paternal grandfather. Was considered a sage and elder of "Mbari Ya Njunu". As for your wife "Mwari Wa Njau" I wish I saw her.
. Mwari wa Mugo (Cucu)	-	Begot my mother, Nduta, Uncle Sam and Francis. Your husband Gatai (from whom I am named) was supposed to be strong and kind - How I longed to see him.
. All My Brothers and Sisters		You are so dear to me, I love you all. Your contribution to my entire self is immense.
. Irungu wa Simeone	-	My all time friend, he introduced me to the literary world.
. "Agaciku a Mbari ya Njunu"	-	This is my traditional orthocentre. I value your principles in deep veneration. Yours is more than conciliation. True progeny of Cikuyu and Mumbi.
. The Inflammed of the World	-	The fangs of Inflammation are sharp and long but with FK compourds we shall all glee and wax robust.

TO THE ENTIRE KAHATO FAMILY, BELIEVERS OF FREEDOM AND JUSTICE, ALL THE INFLAMMED OF THIS COSMOS - HERE YOU ARE;

I WHOLE HEARTEDLY DEDICATE THIS WORK TO YOU ALL.

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TO YOU ALL:

ASANTE MINGI

TABLE OF CONTENTS

	PAGES
DEDICATION	
ACKNOWLEDGEMENTS	
LIST OF TABLES	(i)
LIST OF PLOTS	(ii)
ABSTRACT	(iii)
INTRODUCTION	1
OBJECTIVES	14
MATERIALS & METHODS	15
EXPERIMENTAL	16
RESULTS	22
DISCUSSION	67
PLATES	72
REFERENCES	76

LIST OF TABLES

Table AI - Inhibition of castor oil-induced diarrhoea: A qualitative bioassay for anti-inflammatory activity and its mechanism of action.

Table BI - Effect of castor oil on colonic water flux.

Table BII - Effect of pretreatment with various drugs on castor oil-induced colonic water flux.

Table BIII - Effect of pretreatment of rats on mean rate of castor oil-induced colonic water flux.

Table Co - Calibration absorbance readings for Sodium and Potassium standard curves.

Table CI - Raw data for study Renal aspects - volume of urine, pH changes, level of protein in urine.

Table C2 - Quantitative bioassay for diuretic activity: Effect of drugs on the rate of urine excreation (i.e. variation of urine excreation rate with time).

Table C3 - Evaluation of diuretic effects due to various drugs.

Table C4 - Quantitative bioassay for mineralocorticoid activity: Effect of drugs on Sodium and Potassium output.

Table C5 - Cummulative amount of Sodium produced at each time interval (variation of sodium excreation with time).

Table C6 - Cummulative amount of Potassium produced at each time interval.

Table C7 - Quantitative bioassay for mineralocorticoid activity: Effect of drugs on potassium and sodium excreation rates.

Table C8 - Variation in average rate of sodium excreation with time.

Table C9 - Variation in average rate of potassium excreation with time.

Table C10 - Variation in average cummulative rates of sodium and potassium excreation.

Table C11 - Variation in amount of potassium excreated at each interval with time.

Table C12 - Variation in amount of sodium excreated at each interval with time.

Table C13 - Summary data on Renal Aspects: Simultenous consideration of water and mineral excreation - Data used for the double cummulative plots.

LIST OF PLOTS (GRAPHS)

Plot BI - Effect of castor oil in colonic water flux.

Plot BII - Effect of rat pretreatment with various drugs on castor oil-induced colonic water flux.

Plot BIII - Effect of pretreatment of rats with various drugs on rate of castor oil-induced colonic water flux.

Plot C1 - Volume of urine produced (mls) against time (hours).

Plot C2 - Volume of urine produced against time (after considering the volume due to vehicle as a baseline).

Plot C3 - Cummulative amount of urine excreated against time.

Plot C4 - Average excreation rate of urine against time: Comparison between control rates and those of 4 hours later.

Plot C5 - Cummulative amount of sodium in urine against time.

Plot C6 - Cummulative amount of potassium in urine against time.

Plot C7 - Cummulative amount of sodium produced at each time-interval against time.

Plot C8 - Cummulative amount of potassium produced at each time-interval against time.

Plot C9 - Variation in sodium excreation with time: Comparison of extent of variation between control samples and 4-hour duration samples.

Plot C10 - Variation in potassium excreation with time: Comparing extent of variation between control samples and 4-hour duration samples.

Plot C11 - Cummulative rate of sodium excreation against time (variation of excreation rate with time).

Plot C12 - Cummulative rate of potassium excreation against time (variation of excreation rate with time).

Plot C13 - Cummulative amount of sodium in urine against cummulative amount of urine produced.

Plot C14 - Cummulative amount of potassium in urine against cummulative amount of urine produced.

ABSTRACT

The drugs used conventionally in the treatment of inflammatory states are either steroidal or non-steroidal. The emergence of FK compounds - a series which comprises several novel compounds unique in having bridged the time - honoured gap between steroidals or non-steroidals represents a synthetic feat which commands admiration.

Previous few workers have screened activity of these compounds and the already accrued data suggest that these compounds display potent anti-inflammatory action in the rat and results from castor oil test and Carageenan rat paw oedema testify to these effects.

In pursuit of the mechanism of action of the FKs using "The Delay in Castor oil-induced diarrhoea" (an acute, non-invasive model) and confirmed by the castor oil-induced colonic water flux (an acute invasive model), the results obtained in this present work are suggestive of PG-synthetase inhibition. Qualitative assessment of the results indicate that some of the compounds are more potent than Indomethacin - an established PG synthetase inhibitor.

Consistent with the now widely held and established view that PG synthetase inhibitors show shared side effects namely: gastrointestinal toxicity, nephrotoxicity, inhibition of platelet aggregation and delay in pregnancy and parturition, I embarked on the investigation of the renal side affects of these FK compounds and results obtained displayed varying extents of side effects on water and mineral activity in the kidney, with some causing extreme loss of sodium and potassium while others with potential diuretic side effects.

Further, this work indicates the acute need for more sensitive and direct models to really establish the mechanism of action and the potency of these novel compounds.

Undoubtably, the extent of their side effects needs to be urgently established and the renal data I have presented be confirmed by further experimentation, if they have to achieve any future place in therapy of inflammatory states.

I N T R O D U C T I O N

INTRODUCTION

Castor oil obtained from seeds of Ricinus communis has been recognized and used for centuries as a cathartic (1, 2). The cathartic property is partly due to ricinoleic acid, its hydrolytic product (3a, 3b). A lot of studies have been done with an aim of elucidating the mechanism of diarrhoea due to ricinoleic acid and the effect of this acid and other fatty acids on the intestinal absorption of water, glucose and electrolytes (4 - 20). From these studies the following has been established:

That fatty acids inhibit colonic water and electrolyte absorption (9, 10, 11, 12);

That ricinoleic and other long chain hydroxy-fatty acids cause net colonic water and electrolyte secretion (13, 14, 15);

That the mechanism of diarrhoea due to ricinoleic acid is due to irritation of mucosal cell layers leading to inflammation and consequent release of prostanglandins (16, 18, 19, 20, 142).

The current theory suggests that ricinoleic acid exerts its cathartic effects by serving as an exogenous substrate for the intestinal prostanglandin biosynthesis (19).

Prostanglandins were discovered in 1935 by Goldblatt and Von Euler in 1936, were overhastily named after prostate gland from which it was supposed that they were derived (actually it was the seminal vessicles). The name has persisted to-date although it is now generally known that they occur in almost all the body tissues. Prostanglandins are modified fatty acids (20 carbon, polyoxygenated and unsaturated with cyclopentane ring), and are a result of enzymatic synthesis from Arachidonic acid and membrane bound phospholipids (20, 21, 22a). The biological activity of prostanglandins is very high and their half life is very short. Their measurement in biological fluids and tissues has in order of increasing sensitivity been measured by biological assay, radioimmunoassay and combined gas chromatography - Mass Spectrometry (23, 24, 25, 26, 27).

Prostanglandins are now implicated in many biological mechanisms both physiological and pathological, their effect awesome and showing bewildering diversity. Their effects can only be briefly summarised. On cardiovascular system, PGEs and PGAs are potent vasodilators, PGE, PGF and PGA increase cardiac output. PGI_{22} causes prominent hypotension due to dilation in vascular beds including coronary, renal mesenteric and skeletal muscle (29 - 41).

In the uterus, PGEs and PGFs produce strong contractions of isolated guinea pig uteri in oestrus and diestrus. Strips of human uterus are contracted by PGFs but relaxed by PGA, PGE and PGB. Contractile response is most prominent before menstruation whereas relaxation is greatest at midcycle. Contrastingly, to the invitro behaviour, the human uterus invivo whether pregnant or not is always contracted by PGE_1 PGE_2 and PGF_{2a} (42 - 48).

On GIT, prostanglandins show responses which vary widely with species, segment, type of muscle and the particular prostanglandin. In general longitudinal muscle from stomach to colon is contracted by both PGEs and PGFs while circular muscle generally relaxes to PGEs and contracts to PGFs. Prostanglandins shorten transit time in small intestine and colon. In patients given oral PGs for abortion the common side effects have been diarrhoea, cramps, reflux of bile, nausea and vomiting. PGEs, PGAs and PG_{12} inhibit gastric secretion stimulated by histamine, feeding or hormone gastrin (42, 49, 50, 51, 52, 53).

Current invitro and invivo studies in animals suggest that PGs and cholera enterotoxin may act upon a single intestinal secreatory mechanism. Both PGs and cholera enterotoxin stimulate mucosal adenylcyclase and cause ion transport changes invitro similar to those caused by cAMP and theophylline (54, 55, 56).

On blood PGs and related products exert powerful actions on platelets. PGE_1 and PGD_2 are inhibitors of platelet aggregation in humans. Thromboxanes A_2 (product of Arachidonic acid metabolism) is a powerful inducer of platelet aggregation and platelet release reaction (This action of TXA_2 is sensitive to inhibitory action of Aspirin) (57, 36, 24).

On the kidney, PGEs, PGAs and PG_{12} but not $6ketoPGF_{1a}$ infused directly into renal arteries of dogs increase renal blood flow, provoke diuresis, natriuresis and kaliuresis. These effects of PGs seem to result from a direct action on tubular transport process (Zins, 1975; Dunn and Hodd, 1977, Bolger et al 1978, Hill and Moncada 1979, Grenier and Smith 1978). PGEs inhibit water reabsorption induced by ADH in toad bladder and in rabbit collecting tubules (22b).

On CNS, many stimulant and depressant of PGs have been reported (Horton 1969, 1972, Coseani 1974). Fever is caused by PGE_2 and its release may explain the genesis of pyrogen induced fever and symptoms related to it such as malaise (61).

On the smooth muscles, PGs may contract or relax depending on species, type of PG, endocrine status of the tissue and conditions of the experiment (22b). On afferent nerves and pain perception, PGEs cause pain when injected intradermally and they irritate mucous membranes of the eye and respiratory passages. Release of PGs during inflammatory process thus serves as an amplification system for the pain mechanism (62).

A variety of endocrine tissues respond to PGs. In the rat PGE_1 and PGFs stimulate release of ACTH invivo and PGEs enhances the release of growth hormone invitro. PHF_{2a} has stimulatory effect on secretion of Prolactin, Gonadotrophins and the release of LH and Thyrotropin. Stimulation of adrenal steroid production and insulin release have also been reported (66). The mechanism of luteolysis due to PGF_{2a} is not yet very clear (43, 48, 61 and 63).

Metabolically PGE_1 inhibit the basal rate of lipolysis stimulated by exposure to catecholamines and other lipolytic hormones. PGEs have insulin like effects on carbohydrate metabolism and exert parathormone like effects that result in mobilization of calcium from bone tissue culture (22b, 64, Klein and Raise 1970).

In attempting to seek a mechanism of action for the PGs, workers have postulated the concept of membrane bound receptor and indeed by ligand binding studies receptors of PGE_1, PGE_2, PGF_{2a} have been identified. In many tissues PGs stimulate synthesis of cAMP via activation of adenylate cyclase enzyme (50).

In the platelets PGI_2, PGE_2 and PGD_2 inhibit platelet aggregation by increasing concentrations of cAMP and stimulation of smooth muscle by PGs appears to be associated with depolarization of cellular membranes and the release of bound calcium (Gerald et al, 1976; Smith et al, 1977, Gorman et al, 1978).

The signs and symptoms of the inflammatory process are due in part to prostanglandins which may be released due to mechanical, thermal, chemical, bacterial and other insults. (62 - 80). PGEs and PGI_2 enhance pain producing activity, oedema inducing effect of bradykinnins.

PGs have also been implacated in control of immunological response with PGE_1 regulating functions of B- lymphocytes. It has been suggested that PGs by inhibiting T and B cell functions might facilitate graft acceptance. In sensitised T-lymphocytes PGE inhibits production and release of lymphokines (42, 67).

Therapeutically, PGs (notably PGF_{2a}) have found some use as arbortifacients of choice in midtrimester arbortion, as alternatives to oxytocin for inducing labour at term (69, 45). As alternatives to isopterenol in bronco-asthma, PGs value by inhalation is limited by their irritant effects on the respiratory mucosa (46, 22b, 60).

Oral use to inhibit gastric acid secreation and hence anti-ulcer effect is limited by undesirable side effects such as diarrhoea. The use of PGs (PGI_2 and its analogues may become important in thrombo-embolic disorders (36).

The seventies, did witness a lot of work in the area of pharmacology and therapeutics. It was in June, 1971 when the whole concept of the mode of action of asprin like drugs was changed by the discovery that they inhibit PG- biosynthesis (80, 81).

Presently, the data accumulated on "Inhibition of PG synthesis as a mechanism of action of asprin like drugs" is overwhelming (30 - 114). This theory elaborated by Vane et al is based on the following considerations:

That inflammatory stimuli (be they mechanical, chemical, bacterial, thermal or other insults) induces PG synthesis and release;

That these PGs contribute to the genesis of fever, pain, erythma, oedema plus other signs and symptoms of inflammation;

That asprin like drugs prevent PG production thereby reducing signs and symptoms of the inflammatory process.

To-date there are several NSAID employed in the management and treatment of inflammatory symptoms of all forms of Arthritis including the widespread diseases of rheumatic arthritis, oesteoarthritis and musculoskeltal disorders (152). Thus widely differing NSAID seem to have similar therapeutic action in man namely: reducing fever, pain and inflammation. Further observation that NSAID also share similar side effects namely: gastrointestinal irritation, renal toxicity, inhibition of platelet aggregation, delayed and prolonged parturition strongly indicates that NSAID act by intervention at a single biochemical pathway.

From the foregoing Vane et al were led to theorize and propose that the shared therapeutic effects of the chemically dissimilar NSAID could be accounted for by reduction in that biosynthesis of prostanglandins which accompanies pathological processes. Simultenously, however, any prostanglandins which is necessary for normal physiological processes will also be reduced this accounting for the similar shared side effects of NSAID (23).

From the assembled evidence so far, NSAID inhibits cyclooxygenase activity the mechanism is complex and differs quantitatively among various NSAID. For indomethacin this anti enzyme effect is time dependent, substrate dependent (competitive) and irreversible (most NSAID behave like indomethacin). PG synthetases prepared from different tissues show different sensitivities to NSAID, this property which may reflect a series of isoenzymes explaining the observed differences within the NSAID.

Since several chemically dissimilar NSAID share similar useful therapeutic effects and similar undesired toxic side effects, this strongly indicates PG in those particular physiological and pathological processes. Since the locus between the various physiological and pathological processes is the enzyme. "PG- synthetase", this implies that the "PG synthetase test" described by Piper and Vane stands a better chance of becoming a useful tool for designing new

anti-inflammatory drugs. The effects of NSAID on parturition and platelet aggregation suggests that they could be therapeutically employed in premature labour and thrombosis (36, 45, 69).
NSAID have been tried in dysmenorrhoea with success (114, 115, 116).

The mode of action of NSAID is multifacetated and indeed only partially understood even to-date. These agents appear to interact at a number of points in the complex process of inflammation. So far laboratory models have shown that:

i) Virtually all NSAID inhibit PG biosynthesis by inhibiting cyclooxygenase (this theory is based on sound laboratory and clinical data). The NSAID decrease intrasynovial PG levels thus relieving the pain in arthritis.

ii) NSAID at clinically effective concentrations have been shown to inhibit migration of PMNs and monocytes into inflammed site, further some of the NSAID can inhibit the release of lysosomes from PMNs invitro.

iii) Superoxide anion and hydroxy radical formation are known to accompany damaging enzymes released by phagocytes during inflammation, although short lived these species are very toxic to living tissue. NSAID inhibit formation of the toxic superoxide anions this contributing to their anti-inflammatory action.

iv) Orally administered NSAID have been shown to be present in the fluid of inflammed human knee joints; suggesting direct distribution into inflammed areas. The acidic nature of virtually all NSAID contributes significantly to their rapid absorption into plasma and their being bound to circulating proteins. Further, their lipophilic nature facilitates movements across cell membranes. The NSAID by moving rapidly into the plasma compartments and binding to plasma proteins appear to have the proper physical properties for reaching the inflammed area following oral administration (in fact radiolabelled NSAID have been shown to be preferentially distributed into inflammed tissue). Since NSAID distributes rapidly into synovial joints then these pharmacokinetic property appear to contribute to the clinical activity of NSAID.

v) NSAID have been shown to suppress IgM - rheumatoid factor production by human lymphocytes with rheumatoid arthritis (Lancet 1982: 1 528 - 530; Clinical Immunol. Immunopathol. 1980, 15: 106 - 122).

The situation with steroidal anti-inflammatory drugs (NSAID) is still not well established and at times it is contraversial. The role of PGs in inflammation is well established (70 - 80) and after the discovery that NSAID act at least in part by inhibiting cyclo-oxygenase activity, Vane and other workers were not able to demonstrate such an effect with glucocorticoids (80 - 114). Yet, like the NSAID glucocorticoids are known to inhibit the erythema, oedema and tenderness of inflammation in addition to the effects they have in suppressing chronic inflammation. It seems likely that the action of glucocorticoids is to decrease glucose utilization and to modify the metabolism of lipids and proteins by stimulation of catabolism. The hormones act by stimulation of transcription thus controlling the rate of synthesis of certain key proteins (Thompson and Lipmann, 1974, Metabolism 23: 59).

In several tissues the mechanism of steroid hormone action depends on combination of the steroids with a cytosolic - receptor protein, the translocation of this drug - receptor complex into the nucleus and the inition of protein biosynthesis (124).

The anti-inflammatory effect of the glucocorticoids has not been very properly elucidated. However, a possible mode of action could be due to an inhibition of Phospholipases and by some mechanism still not clear preventing release of PGs formed from arachidonic acid.

Various invitro evidence suggest that steroids block the phospholipase-induced release of Arachidonic acid from cell membrane storage site. The distribution between inhibition of release and PG-synthesis was first postulated by Lewis and Piper 1965 who suggested that steroids inhibited release of arachidonic acid and not PG biosynthesis (101).

Recent work (131a, 131b) has showed that glucocorticoids do not inhibit PG formation in adipose tissue, where the hydrolysis of phospholipids is not the limiting step for the formation of free arachidonic acid.

Danon et al, (1978) were able to show that the glucocorticoids inhibited the PG-biosynthesis by minced renal papilla incubated in tissue culture medium but not in Kreb's buffer and that inhibitors of DNA-dependent RNA-synthesis abolished the inhibitory effect of cortisol i.e. inhibition of PG synthesis by corticosteroids requires protein synthesis (122). This indicates that all organ preparations without protein synthesis could not show inhibitory effect of glucocorticoids. In an elegant series of experiments Flower and Blackwell (124), 132); were able to show that glucocorticoids inhibit release of PG Endoperoxides and TXA_2 in lungs of healthy guinea pigs, whereas they had no influence on the coversion of exogenous arachidonic acid to PG products in the lungs (85).

From further experiments, Flower and Blackwell (124, 132) concluded that dexamethasone is bound to a receptor protein in the cells of the lung. With . continued biosynthesis of protein and RNA, the steroids induced-factor mimics the antiphospholipase effects of these agents. This suggestion was supported by the fact that arachidonic acid in all experiments could abolish the inhibitory effect of glucocorticoids (122, 125, 85) whereas it could not antagonize the effects of Indomethacin or other cyclo-oxygenase inhibitors. These results confirmed the first experiments of Vane (80) that glucocorticoids have no influence on the cyclo-oxygenase activity and the suggestion by Gryglewski (125, 129, 130) that they impare release of PGs by hindering the liberation of arachidonic acid from the membrane phospholips, as Flower (132) was able to demonstrate directly.

From these works it was supposed that an inhibition of phospholipase A_2 activity and by this means a reduced PG - formation are the cause of anti-inflammatory effects of glucocorticoids correllation between anti-inflammatory effects of steroids and their antiphospholipase activity at the isolated guinea pig lung. (Where Dexamethasone blocks phospholipase A_2).

Recent work has elucidated some evidence that glucocorticoids (and not mineralocorticoids) directly inhibit phospholipase A_2 and further that PGs (PGE, $PGF_{2\alpha}$) seem to decrease phospholipase activity thus regulating their own formation in this way (136).

Glucocorticoids have been used in a variety of diseases including: (i) Allergic diseases e.g. Asthma, Hayfever and allergic rhinitis, serum sickness and angioneurotic oedema and anaphylactic reactions, (ii) Glucocorticoids have also found important use in the management of collagen and musculo-skeletal diseases e.g. Rheumatoid Arthritis, osteoarthritis, gout, lupus erythematosus, polyarthritis nodosa, scleroderma, rheumatic fever, (iii) In immunosuppression - glucocorticoids suppress antigen - antibody reactions, (iv) In diseases of the eye, topical applications of steroids - antibiotic combinations are often effective in conjuctivits thus relieving discomfort, (v) In diseases of the skin glucocorticoids are administered intradermally, topically or subdermally. In general glucocorticoids have been considered as life saving due to their extensive use especially so in leukemia, haematologic disorders and tumours, hodgkins and lymphosarcoma and in pulmonary diseases. Glucocorticoids have also been employed in renal diseases e.g. glomerulonephritis, nephrosis hypotension and shock (161).

Toxicity of glucocorticoids results from an overdosage or chronic usage, and is an extension of the effect on physiological and biochemical process in the body. The side effects are a function of time and dosage and may occur after administration of small doses for a long period of time or high doses for a relatively short period. Side effects of glucocorticoids include mostly:

i) Iatrogenic Cushing's Syndrome which is characterised by cushingnoid appearance where there is rounding, puffiness, plethora of the face, shift of fat distribution.

ii) Development of fat malmetabolism leading to obesity.

iii) Carbohydrate intolerance leading to diabetes mellitus.

iv) Development of negative nitrogen balance which leads to muscle weakness, myopathy and osteoporosis.

v) Steroid myopathy - this is characterised by muscular weakness and atrophy especially of the gluteal and thigh muscles.

vi) Osteoporosis occurs as a result of decreased bone matrix formation and increased bone reabsorption.

vii) Skin and skin appendages are affected by chronic glucocorticoid theraphy resulting in thinning, loss of elasticity and plethora of the skin, occurance of acne and acneiform lesions particu-

viii) Wound healing is delayed in patients on chronic, high dose glucocorticoid therapy.

ix) Peptic ulceration - this is one of the most potentially serious side effect of chronic, high dose glucocorticoid therapy. Glucocorticoids are thought to increase gastric acid and pepsin, reduction in mucous protective barrier and changes in gastro-duodenal circulation.

x) Mental symptoms - accompany glucocorticoid therapy especially in patients with rheumatoid arthritis and lupus erythematosus. There are signs and symptoms of mania, depression, agitated depression and suicidal tendencies, overt psychosis may occur.

xi) Hypokalemic, hypochloremic alkalosis, sodium and water retension, some degree of oedema are common in patients on glucocorticoid therapy.

xii) Rapid discontinuation of glucocorticoid therapy may result in rebound of the basic disease process (161).

In view of all these side effects of glucocorticoid therapy they should be administered with caution presumably for short periods under the supervision of the physician. E.g. eye drops containing a steroid are contra-indicated in viral, fungal, T.B. and other infections of the eye including glaucoma. Extended use of topical steroids may cause cataract or increased intra-occular pressure; their use should also be restricted during pregnancy (160).

In pursiut of New NSAID Various models have been used in several laboratories. The model used for the screening and assessing potency of anti-inflammatory agents, are either invivo or invitro. In the majority of the models, they are usually invasive requiring a large number of animal sacrifice, further still the models are either acute or subacute. Interest in PG-synthetase inhibitors has been stimulated by the discovery that Aspirin like drugs block PG biosynthesis - Vane 1971 (80). This discovery provided a new mechanistic concept as well as biochemical approach to the search of new NSAID (137-142).

In vitro models include:

i) Conversion of Arachidonic acid in isolated and perfused guinea pig lungs (described by Piper and Vane 1969).

ii) Effect on tone and motility of rat gastric and uterine muscles. Described by Vane (1957) - uteri from virgin rats are set up in Dujalon solution at 30 degrees centigrade and responses recorded isometrically by means of microdynameter.

(iii) Carageenan induced oedema in hind paw of rat (137, 154).

Invivo models include:

(i) Evaluation prostanglandins synthesis inhibition in rat inflammatory exudate. Described by Higgs (99, 100). The production of an inflammatory exudate is obtained after subcutaneous implantation of polyester sponges soaked in 2% Carageenan and then removed at sacrifice 24 hours later and exudate obtained by squeezing of the sponges.

(ii) Evaluation of PG biosynthesis inhibition in rat gastric mucosa - the presence of PH-like material is assayed on the rat stomach fundus strip (RSS) and PGE_2 used as reference standard.

(iii) Delay in parturation time in rats; described by Chester et al (1972), female Sprague - Dawley rats are mated and when found pregnant are housed individually and test drug given by garage on days 18, 19, 20 of pregnancy, delay in parturation is calculated at the 22 followed by sacrifice and autopsy of the animals 4-20 hours after complete delivery (90).

(iv) Carageenan induced Pleurisy is an excellent acute inflammatory model in which fluid extravasation, leucocyte migration and various biochemical parameters involved in inflammatory response can be measured. (118, 119, 120).

v) ZYMOSAN induced macrophage infiltration into hamstring muscle of rat. This is a chronic model where zymosan is injected into the hamstring muscle of mice. The inflammation characterised by increased muscle weight and concentration of N-Acetyl Glucosamidase. This response is inhibited by local injection of methyl-Predinisolone (a model for chronic muscle inflammation).

vi) Delay in production of castor oil - induced diarrhoea in rats; This noninvasive model of screening anti-inflammatory activity and assessing potency of PG-biosynthesis inhibitors was described by Awouters et al in 1978 (142). In this test rats fasted over night are treated orally by garage with increasing selected doses of test compounds. Then, one hour later the

animals are subjected to a castor oil challenge and presence of characteristic diarrhoeal droppings observed at hourly intervals up to four hours. The absence of any dropping is considered as an inhibitory effect and finally for each compound ED_{50} values at their 95% confidence limits can be calculated.

As will be discussed later, this castor oil test is based on the sound observation that castor oil increases synthesis of PGs in the rat colon (5) and also that it may serve as an endogenous substrate in PG-biosynthesis (19) so that any PG biosynthesis inhibitor should decrease this response and in so doing delay or prevent the castor oil induced diarrhoea. It is this test which was employed in the investigation of FK compounds

FK compounds represent yet another intriguing if not a puzzling feat in the field of anti-inflammatory drugs. The compounds are neither steroidal nor non-steroidal i.e. in attempting to search for newer more potent anti-inflammatory agents Franco Kamau achieved a novel synthetic between steroidal and non-steroidal anti-inflammatory drugs (153). That these compounds do posses some anti-inflammatory activity has been shown (153, 154, 159, present work) and that what is remaining is to:

(i) Critically assess the potency and their more accurate mode of action using more sensitive and more reliable models (radiolabelled "PG-Synthetase test" suggested).

(ii) Assess their side effects (Gastrotoxicity, Nephrotoxicity, on platelet aggregation and on parturation) critically.

(iii) To postulate some kind of SAR which should characterise these FK-bridged putative anti-inflammatory agents.

(iv) To perform pharmacokinetic studies to show whether the compounds are distributed rapidly into the inflammed areas as their future use in therapeutics would depend primarily on enhanced lipophilicity and migration into inflammed sites.

The aim of the present work is to attempt or elucidate the mechanism of action of FK compounds using the castor oil test (142) and to confirm this test using a modified test employed by Beubler & Juan (5).

The study of the side effects due to FK compounds on electrolytes and water were also performed. In studying effects of FK compounds on mineral and water metabolism Frusemide, Hydrocortisone and Dexamethasone were used for comparison (145, 151). In assessing the mechanism of action of the FK series comparison was made with Indomethacin, Dexamethasone in the castor oil test.

OBJECTIVES

Since the discovery in 1971 that NSAID inhibits PG-biosynthesis, it has become fashionable in the search of new anti-inflammatory agents to show that a test compound which inhibits PG-synthetase and thus prevents or decreases PG generation from Arachidonic acid cascade should be a putative anti-inflammatory agent. Further, models which suggest the blockade of PG-synthetase should serve as tools to elucidate the mechanism of action of the putative anti-inflammatory agent. It has already been established that among all the chemically dissimilar NSAID they show similar therapeutic effects and share the same toxici side effects. It follows therefore that investigation of some of the shared side effects e.g. renal toxicity, gastrointestinal irritation etc. could also serve to confirm and help arrive at a therapeutic dose of the putative compound.

From the foregoing, the following have been the objectives of this work:-

(i) Using the albino rat in the bioassay - To find the mechanism of action of a series of FK compounds. These novel synthetic - bridged compounds are assessed using "The Castor oil-induced Diarrhoea Test" and their action compared to the already established potent PG-synthetase inhibitors like Indomethacin. The action of these putative anti-inflammatory agents is also compared to other established potent steroidal anti-inflammatory agents which act probably by a different mechanism (a qualitative bioassay).

(ii) To confirm the mechanism of action of the putative anti-inflammatories by considering the effects of their pretreatment on castor oil-induced colonic water flux (a quantitative bioassay) and comparing this effect with that of established potent anti-inflammatories (Dexamethasone, Indomethacin).

(iii) To investigate the effects of the putative compounds on the renal system. Possible side effects on the renal system were considered by investigating the effects of the compounds on water metabolism (urine excreation), electrolyte excreation, pH changes and levels of glucose and protein in urine. The effects were compared to those of potent diuretic (Frusemide), potent anti-inflammatory agents (Dexamethasone, Indomethacin and Hydrocortisone).

MATERIALS AND METHODS

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	COMPOUND/AGENT/REAGENT	SOURCE/CODE NO.
1.	Castor oil	E.T. Monks & Co.
2.	Sterile disposable syringes	Top Surgical MFG Co. Ltd.
3.	Rats (Albino)	D.O.P. Animal House
4.	Cages	Pharmacology Lab. D.O.P.
5.	Stop watch	Pharmacology Lab. D.O.P.
6.	Urethane	Aldrich Chemical Co. Ltd.
7.	Analytical balance	Sartorius, type 2474, Fabr. Nr. 2506007
8.	Tween 80	I.C.I.
9.	Polyethylene glycol	I.C.I.
10.	Oleic acid	M & B Lab. Chemicals
11.	Sodium Chloride	Analar (Hopkin & Willi)
12.	Anhydride sodium sulphate	M & B Lab. Chemicals
13.	Potassium Chloride	M & B Lab. Chemicals
14.	Barium Chloride	Howse & McGeorge Ltd.
15.	D (+) Glucose	M & B Lab. Chemicals
16.	Sodium Carbonate	E.T. Monks & Co.
17.	Potassium dihydrogen phosphate	Howse & McGeorge Ltd.
18.	Universal Indicator paper	
19.	FK10 INDO, FK12 INDO, FK12 AC, FK12 AC LACT	Synthesized by Mr. F. Kamau, lecturer in Pharm. Chem. Dept. of Pharmacy, U.O.N. Kenya.
20.	Indomethacin	Sample from F. Kamau
21.	Frusemide	Maca Pharmaceuticals
22.	Dexamethasone	Dawa Pharmaceuticals
23.	Flame Photometer	Corning Eel Pat No. 712700
24.	Hydrocortisone	Glaxo East Africa
25.	Homogeniser	
26.	Sodium Hydroxide	General Lab. grade
27.	Sodium dihydrogen phosphate	M & B Analytical Reagent

E X P E R I M E N T A L

EXPERIMENTAL

Preparation of Materials:

1. 10% Castor Oil Emulsion - This was prepared by making a dispersion containing 10 mls castor oil, 88 mls Tyrode solution and 2 mls polyoxyethylene sorbitan mono-oleate (Tween 80). This emulsion is homogenised using a hand homogeniser to ensure complete dispersion. A uniform white viscous emulsion was obtained.
2. "Vehicle" for FK Compounds - This was prepared by mixing polyethylene glycol to a solution containing 0.2% ^{w}/v Tween 80 in water so that the resultant solution contained 4% ^{w}/v polyethylene glycol in 0.2% ^{w}/v Tween 80 in water.
3. FK Solutions - Solutions of FK compounds were prepared by dissoving powders of FK compounds in the vehicle. A few drops of NaOH were sometimes added to facilitate dissolutions. In some cases since the powders did not dissolve completely, they were administered as uniform suspensions.
4. Tyrode Solutions - This was always freshly prepared.
5. Cages - These were cleaned, dried and ordinary white paper placed at the bottom of the cage. Polyethylene paper was not used since this retains urine which may convert otherwise hard faecal matter into diarrhoeal like material and this may interfere with interpretation of quantal responses based on observation of diarrhoea occurence.
6. Flame Photometer - was cleaned calibrated and standards for Potassium and Sodium were prepared, this being necessary for producing a standard (working) curve.

A. EFFECT OF TEST COMPOUNDS ON CASTOR OIL-INDUCED DIARRHOEA:

This is an acute invivo model which is used here as qualitative bioassay for putative anti-inflammatory activity.

Method

(a) Albino rats of both sexes weighing 200 ± 50 grams were starved for 24 hours but given free access to water. The rats were maintained individually in separate cages which had ordinary white paper at the bottom. Drugs were administered as follows:

Indomethacin, Dexamethasone, FK10 INDO, FK12 INDO, FK12 AC, FK12 AC LACT - dose administered was 4 mg/kg. Vehicle, Castor oil emulsion, water administered 1 ml/100g of body weight.

Each rat was treated with the 1 ml/100g of body weight of the suspension of the test substance. The substances were administered orally by gavage and for each compound 6 rats were used for every dose. One hour later, 1 ml of castor oil emulsion was administered by gavage to all the rats (castor oil challenge). 30 minutes after this castor oil challenge, all the cages were inspected for the presence of characteristic diarrhoeal droppings on the white paper, this being repeated at 30 minute-intervals until after about 4 hours. In some cases the duration of experiment was extended up to 12 hours. Lack of characteristic droppings was recorded as a positive result. This indicating protection from diarrhoea at that time.

(b) The same procedure was repeated using a dose of 10 mg/kg for Indomethacin, Dexamethasone, FK10 INDO, FK12 INDO, FK12 AC, FK12 AC LACT. For each compound a total of 6 rats were used. The controls constituted of 12 rats given 1 ml/100g of water only (instead of test compound) and then one hour later subjected to the castor challenge.

B. 1. EFFECT OF CASTOR OIL ON COLONIC WATER INFLUX:

This experiment was done following the method employed by E. Beubler and H. Juan (5). However, in this study of castor oil emulsion was instead of Ricinoleic acid its active component. Castor oil is invivally hydrolysed to give Ricinoleic acid, the component responsible for the irritant carthatic properties attributed to castor oil.

Method

(a) Albino rats of both sexes were starved for 24 hours. They were then anaesthetised using urethane (1.25 g/kg) which was injected intraperitoneally. The rats were then carefully dissected to expose the colon which was cautiously rinsed with 20 mls of warm saline (0.15 M NaCl) using a clean sterile syringe.

30 minutes later, lower end of colon was ligated and then 2 mls of castor oil emulsion was filled into the colon and then the upper end ligated. For the controls the colon was filled with Tyrode solution instead of castor oil emulsion.

After 20 minute-duration, the colon was carefully removed, weighed, emptied by allowing the contents to passively move out through an incision made at one end of the piece of the ligated colon and then reweighed.

(b) The same procedure was repeated, this time allowing for a duration of 60 minutes before removing the colon.

A total of 24 rats were used since for each of the durations, 6 rats were used for each compound (i.e. castor oil emulsion and Tyrode solution).

In recording the results the following relationship was used:-

$$NWT = FC - EC - 2$$

where NWT - Net water transport
FC = Weight of full colon
EC = Weight of empty colon
2g - Initial instillate which is 2 ml since relative density of Tyrode solution is 1.000 and that of emulsion is 0.995.

Positive value for NWT would denote net water secretion into colon (EXORPTION) while negative value would denote net water absorption from colon (INSORPTION). Zero value for NWT would indicate absence of insorption or exorption.

(B) 2. EFFECT OF PRETREATMENT WITH TEST SUBSTANCES ON CASTOR OIL INDUCED COLONIC WATER FLUX

Albino rats of both sexes (200 ± 50 gm) were pretreated with test compounds 48 hours preceding the experiment. Solutions or suspensions of the test compounds were administered intraperitoneally at a dose of 4 mg per kg.

After pretreatment the animals were then starved but given free acess to water for 24 hours. Using urethane 1.25 gm per kg injected intraperitoneally the rats were anaesthetised and carefully dissected to expose the colon. The colon was slowly and cautiously rinsed with 20 ml of warm saline solution (0.15M Nacl). 30 mins. later the colon was instilled with 2 ml of castor oil emulsion after ligating the lower end of the colon. The upper end of colon was then ligated and then the emulsion left in the colon for 20 mins. or 60 mins. according to the duration required. It was crucial to cover the whole dissected area with warm soaked cotton wool to avoid death of the rat due to cold shock (hypothermia).

After the desired duration of time (20 or 60 mins.) the colon was carefully removed by cutting beyond the ligature on both sides, was weighed when full, then allowed to empty passively after making an incision, then afterwards reweighed when empty. As with experiment B1 the relationship NWT = FC - EC - 2 was applied and the results tabulated. (as in Expt. B1)

6 rats were used for each test compount at each duration of time. A for controls 12 animals were used of which 6 were pretreated with normal saline and the other 6 with the vehicle.

(C) RENAL ASPECTS

Effect of Test compounds on the excreation of water, minerals (Na^{+}, K^{+}) was investigated. This was crucial since it can act as a rough guide on the nephrotoxicity side effects of the Test compounds. Other parameters e.g. urine pH, glucose and protein level in urine were also recorded.

METHOD

a) Rats weighing 200 ± 50 gm were starved overnight except for water which they had free acess to. Then, by garage every rat was given a warm water load of 5 ml/100 gm body weight. 6 rats were placed in a meshed cage apparatus which consists of wire

mesh cage for holding the rats, a metallic stand which allows for connection of funnel and cylinder. The funnel was stuffed with cotton wool to prevent entry of debris and any large particulate matter into the cylinder. Any urine collected before one hour was preserved in the refrigerator for later analysis - this was the control sample.

After one hour 1 ml/100 gms solutions/suspensions of test compounds was administered orally by garage. For each of the test compounds and at definite time intervals a sample of urine was taken and the following noted: The volume (cummulative volume), the pH of the urine sample, level of glucose and protein in urine. A sample of 2 ml was stored in the refrigerator for later analysis of mineral content.

b) Standards for potassium chloride and sodium chloride were prepared (as recommended below) and then using a Flame photometer a standard curve was made for KCL and Nacl. The refrigerated urine samples were then diluted 100 times and the absorbance read and recorded.

All the dilutions were done using deionised distilled water.

Preparation of standards for Na^+ and K^+

23 ug of Na^+ is contained in 58.5 ug Nacl

1 ug of Na^+ is contained in 2.5 ug Nacl

Thus standards for following concentrations were as follows: for sodium -

Concentration of Nacl	2.5 ug/ml	5.0 ug/ml	10 ug/ml	20 ug/ml	30 ug/ml
Concentration of Na^+(ug/ml)	1 ugNa^+/ml	2ugNa^+/ml	4ugNa^+/ml	8ugNa^+/ml	12ugNa^+/m

for potassium

39 ug of K^+ is contained in 58.5 ug Kcl

1 ug of K^+ is contained in approx. 2 ug Kcl

Thus standards were considered as follows:

Concentration of Kcl (ug/ml)	2	4	8	10	20
Concentration of K^+ (ug/ml)	1	2	4	5	10

In converting the readings into Milliequivalents per litre (mEq/li), the following 2 formulae were found to be useful (158)

(i) $\text{mEq/litre} = \frac{\text{Weight in mg per litre} \times \text{valency}}{\text{Ionic weight}}$

(ii) $\text{Wt in mg/litre} = \frac{\text{mEq per litre} \times \text{ionic weight}}{\text{Valency}}$

R E S U L T S :

RESULTS

The results, consisting of 3 parts are presented in form of tables and graphs.

PART A: Inhibition of Castor oil - induced diarrhoea: A qualitative bioassay for anti-inflammatory activity and its mechanism of action. The results are summarised in table A1.

PART B: (i) Effect of Castor oil on colonic water flux: The results are summarised in table B1 and plot B1.

(ii) Effect of rat pretreatment with various drugs on castor oil induced colonic water flux: the results are presented in tables BII, BIII and in plots BII and BIII.

PART C: Renal Aspects: In studying renal aspects the following parameters were considers:- Amount and rate of urine production, Amount and rate of sodium and potassium excreation, The pH of u.ine, level o. protein and glucose in urine.

(i) Volume of urine produced; rate of urine production - results summarised in tables CI, CII, CIII and in plots CI, CII, CIII, CIV - This results are supposed quantitative bioassay for diuretic activity.

(ii) Amount of sodium and potassium excreated in urine; rate of potassium and sodium excreation. The results are summarised as follows:

- Amount of sodium and potassium - tables CIV, CV, CVI, CXI, CXII and in plots CV, CVI, CVII, CVIII, CIX, CX.
- Rate of sodium and potassium excreation (variation in excreation rate with time): Tables CVII, CVIII, CIX, CX and in plots CXI, CXII. These results comprise quantitative bioassay on mineralocorticoid activity.

(iii) The variation in urinary pH with time, level of protein and glucose in urine are all contained in table CI which contains "Raw data" on the renal aspects.

(iv) Summary of the data to facilitate quantitative assessment of possible diuretic and mineralocorticoid activity: Table CXIII and plots CXIII and CXIV.

PART B: From table AI - The number of rats showing no diarrhoea are listed under appropriate intervals of time - these rats are said to be protected from castor oil induced diarrhoea. The following is noted from the table:-

(i) The rats given water only showed no diarrhoea i.e. all the 6 rats that were given water only were shown to have no diarrhoea.

(ii) The 6 rats which were not pretreated but given castor oil orally by garage started to diarrhoea progressively after 1.5 hours and were all showing diarrhoea after 3.5 hours - this indicating lack of any protection from the effects of castor oil.

(iii) For the rats pretreated with the vehicle ($4\%^{w}/v$ polyethylene-glycol in $0.2\%^{w}/v$ Tween 80 in water) and then given castor oil an hour later by garage; it is observed that they started showing diarrhoea after 2 hours and that after 4.5 hours all of them were showing diarrhoea.

(iv) The effect of pretreatment with various drugs at two dose levels (4 mg/kg and 10 mg/kg) was also investigated. Qualitatively - It is observed that pretreatment with various drugs one hour before giving a castor oil challenge results in some delay or protection in diarrhoea induced by castor oil. Qualitatively, however, the extent of delay or protection from the castor oil challenge differs from compound to compound and it matches the relative potency of the compound as anti-inflammatory. From table AI - It is clear that of the 2 known potent anti-inflammatory compounds (viz Indomethacin and Dexa.), Indomethacin is more potent in delaying the castor oil induced diarrhoea. This observation is not surprising as the anti-inflammatory effects of Dexamethasone are not mediated by inhibition of the cyclooxygenase like those of Indomethacin (it is to be noted that this model used here is taitor made to suit compounds mediating their effects via cyclooxygenase blockade). The 4 FK compounds studied show activity by this model, qualitatively all the 4 FK compounds delayed and in fact some were shown to completely protect the rats from the diarrhoea due to castor oil challenge. The effect of varying the dose was also studied and qualitatively some effect was shown although it was not very dramatic (see discussion).

PART B:

(i) Effect of Castor oil on colonic water flux (table BI, plot BI)

It is evident that castor oil causes net water flux from blood into colon (Exorption) and that Tyrode solution (the control) causes net water flux into blood from colon (Insorption). With the tyrode solution there is greater absorption of water into the blood when it is left in the colon for 60 mins. than when it is left for 20 mins. With the castor oil secreatory effect of water into colon is greater with 20 mins. duration than with 60 mins. duration (the reason is not clear, in face Beubler and Juan (5) observed the same quantitative difference.) The results obtained by Beubler and H. Juan with Ricinoleic acid are also given below for comparison.

(ii)(a) Effect of pretreatment on castor oil induced colonic water flux (Table BII, plot BII)

From results in table BI it was observed that castor oil causes net secreation of water into the colon, from table BII and plot BII it is clearly evident that this secreatory effect of castor oil is decreased and at times reversed by parenteral administration of the known anti-inflammatory agents (Indomethacin). In fact what emerges is that with all the FK compounds they decrease and in fact reverse the secreatory effects of castor oil, their effect being more pronounced than that of Indomethacin This would appear to suggest that these compounds are potent inhibitors of cyclooxygenase - this view being confirmed by the results obtained in Part A where they were shown to protect or delay the diarrhoea induced by castor oil challenge.

(b) Effect of pretreatement on average rate of castor oil induced colonic water flux - Table BIII, plot BIII

The results obtained and plotted in plot BIII are interesting and consistent with some already known and unknown facts.

(i) With all the compounds (except dexamethasone) the rates at 20 mins. tend towards zero after exposing castor oil in colon for 60 mins.

(ii) The fact that Indomethacin and FK12 AC LACT. have their rates of colonic water flux decaying in a parallel manner may have a bearing to a similar mode of action (or could this have arisen by chance?)

(iii) The qualitative observation that FK12 IND, FK10 IND, FK12 AC have their rates increasing towards zero with time suggest that the mechanism (whichever it is) underlying their action is similar.

(iv) With Dexamethasone the average rate of absorption increases with time (i.e. the rate at 20 mins. exposture is less than that at 60 mins. exposture to castor oil.) This implies that with time the amount of water going into blood (absorption) should increase, that this is so, is borne out by plot BII.

(v) Since with all the compounds (except dexamethasone), the rates tend to go back towards zero (no absorption, no secreation) with time (after about one hour) - what this implies indirectly is some form of similarity in the mode of action. Indeed it is known that inhibition of cyclooxygenase by Indomethacin is time dependent, substrate dependent (competitive), I believe the change in average rate of castor oil induced colonic water secreation towards zero or towards absorption should reflect the extent of cyclooxygenase blockade which results in a fall of prostanglandin level.

<u>Some results obtained by Beubler & Juan (5) for comparison</u>

Compound		Pretreated (Indo)	PGE release	Indo. Pretreated
Control 20 mins.	-0.5 ± 0.04	-0.52 ± 0.08	0.71 ± 0.09ng	0.862 ± 0.12ng
Control 60 mins.	-1.27 ± 0.03	-1.18 ± 0.05	0.6 ± 0.08ng	0.79 ± 0.17ng
Ricinoleic 20 mins.	+0.27 ± 0.03	+0.55 ± 0.08	8.14 ± 1.13	5.81 ± 0.36ng
Ricinoleic 60 mins.	-0.01 ± 0.06	+0.23 ± 0.08	29.51 ± 0.08	10.17 ± 0.75

For these results p 0.01 relative to controls (by paired test) (see discussion).

In assessing the significance of the results obtained in Part B statistical approach was adopted (155, 156) and t-value obtained by the method of paired student "t" test where

$$t = (\bar{x}_A - \bar{x}_B \sqrt{\frac{(N_A + N_B)N_A N_B}{(S_{Y_A}2 + S_{Y_B}2)(N_A N_B)}}$$

- A represents control group while B represents any other test group.
- $\bar{X}_A$ and $\bar{X}_B$ are the mean responses of control and test group respectively.
- N represents the number of observations (in this case N = 6).
- $S_{Y_A}^2$ and $S_{Y_B}^2$ represent the sums of the squares of the deviations from the mean for the control group and the test group respectively.

Thus the t value obtained in this case compared the observations between the control group (castor oil alone, not pretreated) with the other test groups (which were pretreated 48 hours before castor oil administration). At 95% fiducial limits the t value is quoted as equal to 2.5706 (i.e. t 0.05 = 2.5706) when N = 6 and N-1 = 5 (N-1 represents the number of degrees of freedom).

With all the test compounds studied the t value at 20 minutes and at 60 minutes exposure of castor oil in colon, it was found to be higher than 2.5706. This implied that at 95% confidence limits t was greater than 2.5706 and that the probability was greater than 0.05 (i.e. p 0.05) - what this really means is that the results obtained did not arise merely by chance and that the difference in observations of controls and test samples is real and it exists as was statistically borne out.

Part C

(a) The variation in pH, level of protein in urine, absence of glucose in urine and the volume of urine produced at each time interval are all recorded in table CI.

- Variation in pH: For the vehicle, indomethacin, PK12 AC LACT. The pH did not vary much from neutral i.e. in the majority of samples tested the pH was 7, further the total volumes of urine collected after 4 hours for vehicle indomethacin and PK12 AC LACT was 25, 26 and 26.5 mls respectively.
(as was noted in Part B plot BIII, this similarity between indomethacin and PK12 AC LACT appears not to have arisen by chance). With Dexamethasone the pH

was slightly alkaline varying between 8 and 9, for the other FK compounds the pH was distinctly alkaline varying between 9 and 10 - this was also true for Frusemide and Hydrocortisone. The increase in pH reflects disturbance in Acid-Base balance most probably arising from alteration in mineral metabolism.

- Glucose: In all the urine samples tested there was no evidence of glucose in urine, however, the test was only a qualitative one using glucose indicator strips (from KNH). These may not be sensitive enough.

- Protein: The presence of vast amounts of protein in urine in most cases ranging from 200 - 300 mg/100 ml was surprising. The fact that the high level of protein in urine was also found in vehicle sample clearly excludes this effect from being a drug effect. In seeking to explain this apparent disconcordance, it was postulated that high level of protein was due to the enhanced gluconeogenesis arising from acute starving of the animals - That this is true was supported by the observation that after 12 to 24 hours from the onset of the experiment the number of deaths recorded in every cage of 6 animals averaged between 2 and 3 (which is quite high) and further that those remaining were found eating the dead ones - direct support for extreme starvation. In performing this renal experiment one sees no rationale for overnight starving.

b) <u>Volume and rate of urine production</u>

(i) Volume of urine - (plots CI - CIV and tables CI - CII). On purely theoritical grounds one would expect that a drug which increases urine output should produce greater volume of urine and at a faster rate than the vehicle which acts as a control. What emerges from plots CI - CIV is FK12 AC, Dexamethasone, Hydrocortisone and FK10 INDO have smaller volumes and smaller rates than control implying obvious relative to vehicle.

Further with Indomethacin and FK12 AC LACT volumes vary around that of vehicle with slight excreation of water, however, relative to vehicle. With Indomethacin and FK12 AC LACT the terminal slopes in plot CI are shown to be tending to zero. To contrast with this, Frusemide, a potent high ceiling diuretic is attended by a high volume of urine and very high rate of urine excreation. The problem that awaits clarification is the observation that FK12 INDO has a high initial rate of urine excreation (in fact initially higher than Frusemide) but with the terminal slope falling after 3 hours.

From plot CII and CIII it is clear the water loss (enhanced urine excreation) due to FK12 INDO cannot be neglected: plot CIII aptly shows that although the rate of urine production due to FK12 falls faster than that of Frusemide, it is quite clear that the initial incremental production of urine higher than that of Frusemide (this initial incremental increase in urine production due to FK12 INDO is worth further investigation). From plot CIV where the rates of urine production are compared between 0 time (controls) and after 4 hours. It is quite clear that with all the drugs the rates of urine production fall off with time relative to vehicle and that the rates of Frusemide remain still high even after 4 hours.

c) Amount and rate of mineral excreation (Sodium and Potassium)

(i) Amount of Sodium and Potassium produced:
(plot CV - CX). In considering sodium it is observed that FK10, Indomethacin, Dexamethasone are very close to vehicle with only very slight retension. FK12 INDO is close to vehicle with almost negligible excreation. The effects of FK12 AC LACT, Frusemide suggest significant excreation. It is the effects of FK12 AC in causing enormous excreation of sodium that is alarming.

In considering potassium excreation, more or less the same pattern with sodium is replicated with effects of FK10 INDO. Dexamethasone and Indomethacin varying around the vehicle. The effects of Hydrocortisone on potassium excreation are also incorporated. The observation that it causes potassium deficiency is not surprising as it is known that the long term treatment with hydrocortisone results in oedema arising due to sodium retension and potassium depletion.

The effects of Frusemide as a high ceiling diuretic whose mechanism of action is attended by enormous depletion in potassium necessitating K^+ supplementation are clearly borne out by plot CVI. What really is the significant observation that FK12 AC causes significant K^+ loss. Further the terminal slopes of FK12 AC and hydrocortisone are similar.

(ii) In comparing vriation in am unts of minercl production be wen controls and 4 hour duration samples, it is observed (plot C9 and C8) that FK10, Dexamethasone, FK12 INDO, Indomethacin vary only slightly from the vehicle indicating very little mineral-ocorticoid activity. What is really is the observation that is significant is the fact that FK12 AC, Frusemide and Hydrocortisone vary greately from the vehicle with FK12 AC showing greatest variation. It can now be proposed that FK12 AC has mineralocorticoid activity at least relative to Frusemide and Hycrocortisone whose mineralocorticoid effects are well known.

d) Summary on renal aspects

In trying to unify all the data obtained from experiments on renal system table C13 and plots C13 and C14 were made.

In order to meaningfully interprut information contained in plots C13 and C14 (which are very critical) a mathematical model was adopted. The model is a mathematical (strictly, vectorial) approach to the double cummulative plots. With vehicle as the reference point (O, O), cummulative amount of urine is on the X-axis while the cummulative amount of mineral in urine is on the Y-axis. Given at the outset of the "summary data", the theoritical predictions of the model are seen in the plots C13 and C14.

Frusemide a potent diuretic is shown to follow the pattern predicted by the model deviating almost at 45^{0} along the path numbered 1. Thus in the experiments water loss and mineral loss characteristic of the activity of Frusemide were elegantly shown.

The situation of Hydrocortisone with potassium loss and water retension (plot C14) is well understood and documented. It is known that hydrocortisone causes oedema on continued therapy due to sodium retension; potassium loss and water retension (this effect of sodium retension was well observed when the dose of about 100 mg/kg was used). Indeed the plot C14 shows that with time there is almost an about-turn from water loss to water retension this contributing to the well known side effects.

With Dexamethasone - the very slight mineral and water retension (following path f) are well documented and elegantly displayed in plots C13 and C14. It is known that Dexamethasone, a very potent anti-inflammatory agent has only very slight mineralocorticoid (155).

FK Compounds

- FK10 INDO - from plots C13 and C14, it appears that this compound causes only slight water retension and that mineral activity is even smaller than that due to Dexamethasone. The fact FK10, Indomethacin and Dexamethasone have quantitatively very small mineral and water activity, revolving around the vehicle should be noted and perhaps optimised.
- FK12 IND - for both plots C13 and C14, it is clear that the compound causes adequate water loss which is unattended by mineral loss (assuming other factors constant - could this properly be useful in the clearing of the oedema that accompanies inflammation or is dangerous as a side effect?)
- FK12 AC LACT - this compound has significant water lossing effect which is attended also by significant mineral loss. The combination of mineral loss attended by an equivalent amount of water loss indicate some diuretic potential which urgently calls for the need of critical confirmation by further experimentation and

unequivocal methodology. (If this were found to be true then this compound would be limited as an anti-inflammatory due to side effects arising due to mineral loss and water loss.

. PK12 AC - this compound shows peculiar property of causing extreme mineral loss (much more greater than that of Frusemide with respect to sodium). What is more alarming is the observation that despite the extreme mineral loss the amount of urine is very small and does not change much with time. If all factors were taken constant and this data alone used for evaluation, then PK12 AC could have its use limited due to the serious mineral loss accompanying its use.

INHIBITION OF CASTOR OIL-INDUCED DIARRHOEA: A QUALITATIVE BIOASSAY FOR ANTINFLAMMATORY ACTIVITY AND ITS MECHANISM OF ACTION

(TABLE A1)

COMPOUND	Number of Rats Showing No Diarrhoea	TIME INTERVAL (HOURS)									
	Dose	1	1½	2	2.5	3	3.5	4	4.5	5	12
WATER (1 ml/100gms)		6	6	6	6	6	6	6	6	6	6
CASTOR OIL 1 ml/100 body weight		6	4	4	2	1	0	0	0	0	2
INDOMETHACIN PRETREATED	4 mg/kg	6	6	5	4	4	4	4	4	3	2
DEXAMETHASONE PRETREATED	4 mg/kg	6	6	5	5	4	4	4	3	3	3
VEHICLE(ONLY) PRETREATED	1 ml/100g	6	6	5	4	2	2	1	0		
FK10 IND PRETREATED	4 mg/kg	6	6	6	6	5	5	5	5	4	
FK12 AC PRETREATED	4 mg/kg	6	6	6	6	6	6	6	6	6	1_3dead
FK12 AC LACT PRETREATED	4 mg/kg	6	6	6	6	6	6	5	5	4	
FK12 IND PRETREATED	4 mg/kg	6	6	6	6	5	5	5	5	5	2
INDOMETHACIN PRETREATED	10 mg/kg	6	6	6	5	5	4	4	4	4	3
DEXAMETHASONE PRETREATED	10 mg/kg	6	6	6	6	5	5	5	4	4	4
FK10 IND PRETREATED	10 mg/kg	6	6	6	6	6	6	6	5	5	3
FK12 AC PRETREATED	10 mg/kg	6	6	6	6	6	6	6	6	6	6
FK12 AC LACT PRETREATED	10 mg/kg	6	6	6	6	6	6	5	5	5	2
FK12 IND PRETREATED	10 mg/kg	6	6	6	5	4	4	4	4	4	1

EFFECT OF CASTOR OIL ON COLONIC WATER FLUX (TABLE - BI)
EXPT 2A

SUBSTANCE	INTERVAL	INDIVIDUAL RESPONSES			MEAN ± S.E.M.
TYRODE CONTROLS	20 MIN.	0.03 0.15	0.01 0.19	0.14 0.04	0.02 ± 0.05
TYRODE CONTROLS	60 MIN.	0.68 0.54	0.75 0.43	0.675 0.62	0.616 ± 0.036
CASTOR OIL	20 MIN.	0.80 0.752	0.85 0.70	0.874 0.77	0.791 ± 0.026
CASTOR OIL	60 MIN.	0.155 0.32	0.57 0.39	0.60 0.36	0.399 ± 0.068

EFFECT OF PRETREATMENT WITH VARIOUS DRUGS ON CASTOR OIL INDUCED DIARRHOEA EXPERIMENT 2B (TABLE - BII)

SUBSTANCE	INTERVAL	INDIVIDUAL RESPONSES			MEAN ± S.E.M.	t-VALUE	PROBABILITY	SIGNIFICANCE
INDOMETH	20 MIN.	0.54 0.48	0.57 0.51	0.60 0.45	0.525±0.023	11.0	P>0.05	S
DEXAMETH	20 MIN.	0.05 −0.09	0.2 −0.37	−0.04 −0.07	0.053±0.077	8.66	P>0.05	S
FK10 IND	20 MIN.	−0.23 −0.37	−0.15 −0.14	−0.19 −0.20	−0.213±0.005	11.89	P>0.05	S
FK12 IND	20 MIN.	−0.18 −0.12	−0.13 −0.19	−0.15 −0.09	−0.1633±0.016	5.94	P>0.05	S
FK12 AC	20 MIN.	−1.05 −0.87	−1.2 −0.7	−0.9 −1.1	−0.97±0.111	23.68	P>0.05	S
FK12ACLACT	20 MIN.	0.31 0.49	0.26 0.23	0.56 0.34	0.353±0.054	19.26	P>0.05	S
INDOMETH	60 MIN.	0.20 0.36	0.42 0.28	0.23 0.31	0.3 ± 0.037	2.573	P>0.05	S
DEXAMETH	60 MIN.	−0.16 −0.18	−0.27 −0.25	−0.21 −0.30	−0.228±0.022	8.799	P>0.05	S
FK10 IND	60 MIN.	0.15 0.25	0.19 0.31	0.20 0.33	0.238±0.029	4.642	P>0.05	S
FK12 IND	60 MIN.	−0.09 0.17	0.11 0.22	0.13 0.24	0.16 ± 0.018	7.572	P>0.05	S
FK12 AC	50 MIN.	−0.03 0.06	−0.02 0.10	0.04 0.12	0.042±0.027	7.196	P>0.05	S
FK12 AC LACT.	60 MIN.	−0.03 −0.14	−0.11 −0.20	−0.13 −0.09	−0.167±0.033	17.056	P>0.05	S

. at 95% CONFIDENCE INTERVAL t = 2.5706
(i.e. $t_{0.05}$ = 2.5706 by paired students tests)

. Doe was 4mg/kg −ve value indicate Net water absorption from colon
+ve value indicate Net water secreation into colon

. S - Significant.

EFFECT OF PRETREATMENT OF RATS ON MEAN RATE OF CASTOR-OIL INDUCED COLONIC WATER FLUX (TABLE - BIII)

COMPOUND	INTERVAL	MEAN RATE (mls/min)
CONTROL (CASTOR OIL ONLY)	20 (Min)	3.995×10^{-2}
INDOMETHACIN	20 (Min)	2.625×10^{-2}
DEXAMETHASONE	20 (Min)	-0.265×10^{-2}
FK10 IND	20 (Min)	-1.065×10^{-2}
FK12 IND	20 (Min)	-0.7165×10^{-2}
FK12 AC	20 (Min)	-4.85×10^{-1}
FK12 AC LACT	20 (Min)	1.765×10^{-2}
CONTROL (CASTOR OIL ONLY)	60 (Min)	0.665×10^{-2}
INDOMETHACIN	60 (Min)	0.5×10^{-2}
DEXAMETHASONE	60 (Min)	-0.38×10^{-2}
FK10 IND	60 (Min)	0.3966×10^{-2}
FK12 IND	60 (Min)	0.2666×10^{-2}
FK12 AC	60 (Min)	0.07×10^{-2}
FK12 AC LACT	60 (Min)	-0.278×10^{-2}

NB: For each compound a dose of 4 mg/kg body weight was used.

ABSORBANCE READINGS FOR STANDARD CURVE

(TABLE - C)

CONCENTRATION OF Na^+	ABSORBANCE I	ABSORBANCE II	ABSORBANCE AVERAGE	CONCENTRATION OF K^+	ABSORBANCE I	ABSORBANCE II	ABSORBANCE AVERAGE
1 mg/ml	8	8	8	2.5 mg/ml	16	16	16
2 mg/ml	19	19	19	5 mg/ml	27	27	27
4 mg/ml	34	35	34.5	10 mg/ml	49	49	49
8 mg/ml	58	59	58.5	15 mg/ml	71	71	71
10 mg/ml	72	72	72	20 mg/ml	87	87	87
15 mg/ml	100	100	100	25 mg/ml	100	100	100

The galvanometer deflections (Absorbance) were used to construct a working curve (the standard curve). The absorbance of controls and samples of urine collected at 10, 20, 30, 60, 90, 120, 150, 240 mins. and 24 hours were determined from this standard curve.

TABLE C1

COMPOUND		CONTROL URINE	10 Min.	30 Min.	60 Min.	90 Min.	120 Min.	150 Min.	240 Min.	300 Min.	24 Hrs.	No. of Deaths Recorded Between 12-24 Hrs.
VEHICLE	Vol. of Urine		2.0	6.5	11.5	16.5		23	25		54	2
	PH	7.5	7	7	7	7		7	7		80	
	Protein	++	++	+++	++	+		++	+		+++	
INDOMETHACIN (4mg/kg)	Vol. of Urine	3.5	4.0	5.5	10.5	19		24	26		36	1
	PH	7	7	7	7	7		7	7		8	
	Protein	++	+++	+++	++	+		++	+		++	
DEXAMETHASONE (4mg/kg)	Vol. of Urine		2.5	5.5	8.5	14.5		17	20		60	
	PH	8.0	8.0	8.0	8.0	8.0		7	8.0		9.0	
	Protein	+++	+++	+++	++	++		+++	+++		++	
HYDROCORTISONE (4mg/kg)	Vol. of Urine	7.0		9.0	10.5	14	17	17.5	18		28	3
	PH	10		9.0	10	9	10	10	9		10	
	Protein	+++		++	++	++	+++	+++	+++		++	
HYDROCORTISONE (100mg/kg)	Vol. of Urine	5 ml		10.5	11.5	16.5	21.5	21.5	25		34	4
	PH	9		10	9.0	8.0	9.0	10	10		10	
	Protein	+++		++	++	+++	+++	++	++		++	
FRUSEMIDE (4mg/kg)	Vol. of Urine	5		13	19	24	28	30	33.5	33.5		
	PH	9.0		10	10	10	10	9.0	10	10		
	Protein	+++		+++	+++	+++	+++	+++	+++	++		
FK10 INDO (4mg/kg)	Vol. of Urine	7.5	9.0	9.5	13.5	14.5	14.5	16.5	17.5		32	4
	PH	9.5	9.0	10	10	10	9	8	10		9	
	Protein	+++	+++	+++	+++	++	++	++	++		++	
FK12 AC INDO (4mg/kg)	Vol. of Urine	6.5	13.5	15	20	25	27	27.5	29		46	2
	PH	10	10	10	8.5	9.0	7.0	8.0	8.0		9.0	
	Protein	+++	+++	+++	+++	++	++	++	++		+++	
FK12 AC MeOH (4mg/kg)	Vol. of Urine	5		9	10	16	20 ml	21	23.5		29	1
	PH	9.5		10	10	10	10	8.0	10		10	
	Protein	+++		+++	+++	++	+++	+++	+++		+++	
FK12 AC LACT (4mg/kg)	Vol. of Urine	8	8.5	12.5	19.0	20	23	23.5	26.5		41	2
	PH	7	7	7	7.5	8.0	7.0	7	7		7.0	
	Protein	++	++	+++	++	++	+++	++	+++		+++	

NOTES:

- Frusemide from Nucks Pharmaceuticals (Purity 99%)
- Protein & Glucose was determined by using Glucostrips (from K.N.H.)

KEY FOR PROTEIN:

+ = 30 mg/100 ml, ++ = 100 mg/100 ml, +++ = 300 mg/100 ml

- PH determined by using Universal Indicator paper.
- No Glucose was detected in urine.
- Vehicle is 4% w/v Polyethylene Glucolin 0.2% w/v Tween 80 in water.

QUANTITATIVE BIOASSAY FOR DIURETIC ACTIVITY: EFFECT OF DRUGS ON RATE OF URINE EXCREATION

(TABLE - CII) (VARIATION OF EXCREATION RATE OF URINE WITH TIME)

COMPOUND	DOSE mg/kg		CONTROL	30 MIN	60 MIN	90 MIN	150 MIN	240 MIN	AVERAGE RATE 4 HOURS AFTER CONTROL
			0	0.22	0.167	0.167	0.11	0.022	0.1372
VEHICLE	1 ml/100g	E.O.V.S.	0	0	0	0	0	0	0
			0.058	0.067	0.167	0.28	0.083	0.022	0.1233
INDOMETHACIN	4	E.O.V.S.	0.058	-0.153	0.0	0.113	-0.027	0	-0.013[illegible]
			0	0.183	0.1	0.2	0.042	0.033	0.1112
DEXAMETHASONE	4	E.O.V.S.	0	-0.037	-0.07	0.033	-0.068	0.011	-0.026
			0.12	0.067	0.05	0.12	0.0583	0.0056	0.06018
HYDROCORTISONE	4	E.O.V.S.	0.12	-0.153	-0.12	-0.047	-0.052	-0.0164	-0.07762
			0.1	0.15	0.033	0.167	0.083	0.05	0.0966
HYDROCORTISONE	100	E.O.V.S.	0.1	-0.07	-0.134	0	-0.027	0.028	-0.0406
			0.08	0.27	0.2	0.167	0.1	0.038	0.155
FRUSEMIDE	4	E.O.V.S.	0.08	0.05	0.033	0	-0.11	0.016	0.0178*
			0.13	0.067	0.133	0.033	0.033	0.011	0.0556
FK10 IND	4	E.O.V.S.	0.13	-0.153	-0.036	-0.134	-0.077	-0.011	-0.0818
			0.11	0.28	0.17	0.167	0.042	0.017	0.1352
FK12 IND	4	E.O.V.S.	0.11	0.06	0.003	0	-0.068	-0.005	-0.002
			0 08	0.133	0.033	0.2	0.083	0.028	0.0954
FK12 AC	4	E.O.V.S.	0.08	-0.09	-0.134	0.033	-0.027	0.006	-0.0418
			0.133	0.15	0.25	0.033	0.0583	0.033	0.10466
FK12 AC LACT	4	E.O.V.S.	0.133	-0.07	0.083	-0.134	-0.052	0.011	-0.03234

E.O.V.S. - Effects of Vehicle Substracted.

- Negative sign indicates relative retension of water as compared to vehicle.
- Rate of urine excreation in mls per minute.

*Average rate of urine excreation remains +ve even 4 hours after drug administration.

EVALUATION OF DIURETIC EFFECTS DUE TO VARIOUS DRUGS

(TABLE - CIII)

COMPOUND	Average rate of urine production mls/min / Diuretic Index	INTERVAL (Minutes) 30	60	150	Average D.I.
INDOMETHACIN	A.R.O.U.P.	0.075	0.167	0.083	
	D.I.	0.333	1.00	0.77	0.701
VEHICLE	A.R.O.U.P.	0.225	0.167	0.108	
	D.I.	1.00	1.00	1.00	1.00
DEXAMETHASONE	A.R.O.U.P.	0.15	0.10	0.042	
	D.I.	0.67	0.60	0.39	0.553
HYDROCORTISONE	A.R.O.U.P.	0.067	0.05	0.058	
	D.I.	0.28	0.30	0.54	0.373
FRUSEMIDE	A.R.O.U.P.	0.27	0.20	0.1	
*	D.I.	1.2	1.20	0.93	1.11
FK10 INDO	A.R.O.U.P.	0.025	0.133	0.033	
	D.I.	0.11	0.8	0.31	0.406
FK12 IND	A.R.O.U.P.	0.075	0.167	0.042	
	D.I.	0.33	1.0	0.39	0.083
FK12 AC	A.R.O.U.P.	0.133	0.033	0.083	
*	D.I.	0.59	1.20	0.77	0.853
FK12 AC LACT	A.R.O.U.P.	0.20	0.22	0.11	
	D.I.	0.89	1.32	1.02	1.046

NOTES:

i) A.R.O.U.P. = Average rate of urine production in mls/min and is obtained by evaluating

$$\frac{\text{Amount of urine produced over some time interval (mls)}}{\text{The time interval (mins)}}$$

ii) D.I. = Diuretic Index which is obtained by evaluating

$$\frac{\text{Average rate of urine production by Test Compound}}{\text{Average rate of urine production by vehicle}}$$

- For each of the compounds a dose of 4 mg/kg is used and the effects at 30 min., 60 min. and 150 min. considered.

iii) *Significant diuretic effects are observed
- with Frusemide at 30 and 60 mins. interval
- with FK12 AC LACT at 60 and 150 mins. interval
- with FK12 AC MeOH at 60 mins. interval (see discussion)

QUANTITATIVE BIOASSAY FOR MINERALOCORTICOID ACTIVITY: EFFECT OF DRUGS ON SODIUM & POTASSIUM OUTPUT

(TABLE - CIV)

COMPOUND	DOSE mg/kg	CONTROL		30 MIN		60 MIN		90 MIN		120 MIN		150 MIN		240 MIN		300 MIN		24 HOURS	
		Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+
VEHICLE	1 ml/ 100g	4.565	2.97	4.04	3.41	3.57	2.97	2.83	3.85			2.04	2.31	1.00	1.62			14.78	13.85
INDOME-THACIN	4	2.130	4.15	3.48	4.7	0.87	1.72	1.09	1.79			0.57	1.28	0.70	1.67			4.35	7.77
DEXAME-THASONE	4	2.304	1.72	2.70	2.38	3.35	2.7	1.43	1.36			4.70	2.13	2.1	1.79			10.43	10.25
HYDROCO-RTISONE	4	17.39	25.13	30.87	41.54	9.87	25.13	9.57	17.44	7.83	14.9	8.04	17.44	4.57	6.92			4.04	8.13
HYDROCO-RTISONE	100	8.826	6.15	10.30	7.82	16.09	24.3	7.83	5.54	7.61	5.4	9.0	7.08	12.61	10.26			8.70	8.97
FRUSE-MIDE	4	24.35	40	13.91	34.1	9.13	67.18	8.18	20.77	7.83	13.3	12.61	26.67	8.48	19.23	8.70	15.9		
FK10 IND	4	1.61	4.18	2.26	4.95	2.30	5.64	2.61	5.13	1.17	2.56	1.17	3.38	1.61	6.23			8.13	4.15
FK12 IND	4	1.83	3.08	7.13	9.05	6.0	6.74	2.9	4.62	2.22	3.33	1.87	4.18	1.17	3.08			8.26	18.97
FK12 AC	4	41.74	33.33	31.30	30.77	21.74	20.26	12.83	12.31	7.96	6.92	8.70	8.03	6.83	6.41			5.04	6.82
FK12 AC LACT	4	9.26	7.18	9.22	9.05	5.35	7.26	3.57	5.54	3.48	2.77	2.91	8.41	1.22	2.77			3.57	6.85

NB: . Value for concentration of Na^+ and K^+ in urine computed per rat

. 6 rats were used weighing 200 ± 50 gms.

. The values of concentration were obtained after plotting a standard curve.

. Concentration of K^+ and Na^+ is given in Milliequivalents per litre x 10^{-2} i.e. (mEq/litre x 10^{-2})

CUMMULATIVE AMOUNT OF SODIUM PRODUCED AT EACH TIME INTERVAL

(TABLE - CV) (VARIATION IN SODIUM EXCREATION WITH TIME AFTER DRUG ADMINISTRATION)

COMPOUND	DOSE mg/kg	CONTROL	30 MIN	60 MIN	90 MIN	150 MIN	240 MIN	
VEHICLE	1 ml/100gm	0	0	0	0	0	0	
INDOMETHACIN	4	-2.44	-0.57	-2.71	-1.75	-1.48	-3.04	
DEXAMETHASONE	4	-2.26	-1.34	-1.16	-2.54	1.53	-0.04	
HYDROCORTISONE	4	12.83	26.34	4.87	5.31	4.36	1.93	*
HYDROCORTISONE	100	4.23	6.23	12.89	4.56	5.13	9.78	
FRUSEMIDE	4	19.79	9.88	5.57	5.36	5.80	2.71	*
FK10 IND	4	-2.56	-1.79	-1.28	-0.23	-0.88	0.61	
FK12 IND	4	-2.74	3.09	2.43	0.07	0.18	0.52	
FK12 AC	4	37.2	27.29	18.19	10.03	5.95	5.13	*
FK12 AC LACT	4	4.7	5.19	1.79	0.75	1.45	0.80	

-ve sign indicates that amount of sodium at that interval was smaller than that due to vehicle.

*the effects of Hydrocortisone, Frusemide and FK12 AC are significant (see discussion).

CUMMULATIVE AMOUNT OF POTASSIUM PRODUCED AT EACH TIME INTERVAL

(TABLE - CVI) (VARIATION IN POTASSIUM EXCRETION WITH TIME AFTER DRUG ADMINISTRATION)

COMPOUND	DOSE mg/kg	CONTROL	30 MIN	60 MIN	90 MIN	150 MIN	240 MIN
VEHICLE	1 ml/100gm	0	0	0	0	0	0
INDOMETHACIN	4	1.18	1.29	-1.25	-2.06	-1.03	0.05
DEXAMETHACIN	4	-1.25	-1.03	-0.27	-2.95	-0.64	-0.29
HYDROCORTISONE	4	22.16	38.13	22.16	13.59	17.67	7.84
HYDROCORTISONE	100	3.18	4.41	21.33	1.69	4.91	8.78
FRUSEMIDE	- 4	37.03	30.69	44.27	16.98	31.89	25.14
FK10 IND	4	1.21	1.54	2.67	1.28	3.64	7.18
FK12 IND	4	0.11	5.64	3.77	0.77	3.16	2.75
FK12 AC	4	30.36	27.39	17.32	8.15	10.80	9.87
FK12 AC LACT	4	4.21	5.64	4.29	1.69	8.87	3.92

QUANTITATIVE BIOASSAY FOR MINERALOCORTICOID ACTIVITY: EFFECT OF DRUGS ON POTASSIUM & SODIUM EXCREATION RATE (TABLE - CVII)

COMPOUND	DOSE mg/kg	CONTROL		30 MIN		60 MIN		90 MIN		120 MIN		150 MIN		240 MIN		300 MIN		24 HOURS	
		Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+
VEHICLE	1 ml/ 100g	7.6	4.92	-1.8	1.5	-1.6	-1.47	-2.5	2.93			-1.32	2.6	-1.7	-0.77			1.15	1.02
INDOME-THACIN	4	3.55	6.92	4.5	1.8	-8.7	-9 93	0.73	0.23			-0.9	-0.85	0.22	0.43			0.3	0.51
DEXAME-THASONE	4	3.84	2.9	1.32	2.2	2.3	1.06	-7.1	-4.5			11.0	1.28	-4.3	-0.38			0.7	0.71
HYDROCO-RTISONE	4	29	42	44.9	54.7	-70	-54.7	-1.0	-25.6	-5.8	8.5	0.7	8.47	-5.8	-11.7			-0.44	0.10
HYDROCO-RTISONE	100	14.7	10.3	4.9	5.56	19.3	54.9	-27.5	-62.5	-0.73	-0.47	4.6	5.6	6.0	3.53			-0.33	0.11
FRUSE-MIDE	4	40.6	66.6	-35	-19.7	-16	110.3	-3.2	-154.7	-1.2	-24.9	16	44.6	-7.0	-8.3	0.37	-5.6		
FK10 IND	4	2.68	7	2.2	2.57	0.13	2.3	1.03	-17.1	-4.8	-8.6	0	2.73	-0.7	3.2			0.54	-0.17
FK12 IND	4	3.1	5.13	17.7	19.9	3.8	-7.7	-10.3	-7.1	-2.3	-4.3	-1.2	2.83	-1.2	-1.22			0.6	1.32
FK12 AC	4	69.6	55.6	-35	-11.7	-32	-35	-30	-26.5	-16.2	-16.0	2.5	3.7	-3.12	-1.8			-0.15	0.034
FK12 AC LACT	4	15.4	12.0	-0.13	6.23	-13	-5.17	-6.0	-5.73	-0.3	-9.2	-2.0	18.8	-2.8	-6.3			0.2	0.34

NB: . Average rate of excretion of Sodium and Potassium computed per rat.

. Units are in Milliequivalents/litre/min $\times 10^{-2}$ (i.e. mEq $li^{-1} min^{-1} \times 10^{-2}$).

. A negative sign indicates relative decrease in excreation rate.

VARIATION IN AVERAGE RATE OF SODIUM EXCREATION WITH TIME

(TABLE - CVIII)

COMPOUND	DOSE mg/kg		CONTROL	60 MIN	150 MIN	240 MIN	AVERAGE RATE AFTER 4 HOURS
			7.6	-1.7	-1.87	-1.80	-1.79
VEHICLE	1 ml/100gm	E.O.V.S.	0	0	0	0	0
			3.55	-2.1	-0.77	-0.83	-1.23
INDOMETHACIN	4	E.O.V.S.	-4.05	-3.8	1.10	0.97	0.56
			3.84	1.81	1.90	0.644	1.45
DEXAMETHASONE	4	E.O.V.S.	-3.76	2.70	3.77	1.16	3.24
			29.0	-12.6	-6.13	-6.2	-8.[illegible]
HYDROCORTISONE	4	E.O.V.S.	21.4	-10.9	-4.28	-4.4	-6.51
			14.7	12.1	-2.9	1.1	3.43
HYDROCORTISONE	100	E.O.V.S.	6.7	13.8	-1.05	2.9	5.22
			40.26	25.5	-3.5	-7.73	-12.24
FRUSEMIDE	4	E.O.V.S.	32.7	-23.8	-1.65	-5.93	-10.45
			2.68	1.17	-0.65	-0.36	0.053
FK10 IND	4	E.O.V.S.	-4.92	2.87	1.20	1.44	1.843
			3.10	6.95	-1.71	-0.18	1.67
FK12 IND	4	E.O.V.S.	-4.5	7.80	0.14	1.62	3.46
			69.6	-33.5	-19.3	-18.97	-23.92
FK12 AC	4	E.O.V.S.	62.0	-31.8	-17.50	-17.20	-22.13
			15.4	-6.57	-3.72	-4.04	-4.78
FK12 AC LACT.	4	E.O.V.S.	7.8	1.83	-1.87	-2.24	-2.99

*Degree of variation in rates of excreation between controls and the other samples gives an indication of the effect of drug on mineral metabolism.
Thus Frusemide, Hydrocortisone and FK12 AC have significant mineralocorticoid effects.

*E.O.V.S. - Effects of Vehicle Substracted.

VARIATION IN AVERAGE RATE OF POTASSIUM EXCREATION WITH TIME

(TABLE - CIX)

COMPOUND	DOSE mg/kg		CONTROL	60 MIN	150 MIN	240 MIN	AVERAGE RATE AFTER 4 HOURS
			4.9	0.02	1.85	0.54	0.803
VEHICLE	1 ml/100gm	E.O.V.S.	0.0	0.0	0.0	0.0	0
			6.9	-4.1	-1.57	-0.57	-2.08
INDOMETHACIN	4	E.O.V.S.	2.0	-4.12	-3.42	-1.11	-2.883
			2.9	1.63	0.53	-0.46	0.213
DEXAMETHASONE	4	E.O.V.S.	-2.0	1.61	3.77	-1.0	-0.59
			42.0	0	-2.12	-6.90	-3.01
HYDROCORTISONE	4	*E.O.V.S.	37.1	-0.02	-4.28	-7.44	-3.813
			10.3	30.23	-6.8	-1.64	7.26
HYDROCORTISONE	100	E.O.V.S.	5.4	30.21	-1.05	-2.18	6.457
			66.6	45.5	-22.4	-15.40	2.57
FRUSEMIDE	4	*E.O.V.S.	61.7	45.48	-1.65	-15.94	1.767
			7.0	2.44	-5.13	-0.97	-1.22
FK10 IND	4	E.O.V.S.	2.1	2.42	1.20	-1.51	-2.023
			5.13	6.1	-0.62	-0.92	1.52
FK12 IND	4	E.O.V.S.	0.23	6.08	0.14	-1.46	0.717
			55.6	-23.4	-16.1	-8.95	-16.15
FK12 AC	4	E.O.V.S.	50.7	-23.42	-17.5	-9.49	-16.95
			12.0	0.13	1.0	-2.7	-0.52
FK12 AC LACT.	4	E.O.V.S.	7.1	0.11	-1.87	-3.24	-1.323

E.O.V.S. - Effects of Vehicle Substracted.

*Effects of FK12 AC, Frusemide and Hydrocortisone on Potassium excreation are significant.

QUANTITATIVE BIOASSAY FOR MINERALOCORTICOID EFFECTS:

VARIATION IN RATES OF Na^+ AND K^+ EXCREATION WITH TIME (CUMMULATIVE RATES CONSIDERED)

(TABLE - CX)

	DOSE mg/kg	CONTROL		60 MIN		150 MIN		240 MIN	
		Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+
VEHICLE	1 ml/100gm	0	0	0	0	0	0	0	0
INDOMETHACIN	4	-4.05	2.0	-7.85	-2.12	-6.75	-5.54	-5.78	-6.65
DEXAMETHASONE	4	-3.76	-2.0	-1.06	-0.39	2.71	3.38	3.87	2.38
HYDROCORTISONE	4	21.4	37.1	10.5	37.08	6.22	32.80	1.82	25.36
HYDROCORTISONE	100	6.7	5.4	20.5	35.01	19.45	34.56	22.35	32.38
FRUSEMIDE	4	32.7	61.7	8.9	107.18	7.25	105.53	1.32	89.59
FK10 INDO	4	-4.92	2.10	-2.05	4.52	-0.85	5.72	0.59	4.21
FK12 INDO	4	-4.5	0.23	3.3	6.31	3.44	6.45	5.06	4.99
FK12 AC	4	62.0	50.7	30.2	27.28	12.7	9.78	-4.5	0.29
FK12 AC LACT.	4	7.8	7.1	9.63	7.21	7.76	5.34	5.52	2.1

Rates given in MEq $li^{-1} min^{-1} \times 10^{-4}$

-ve sign indicate a relative decrease in rate with respect to vehicle.

VARIATION IN AMOUNT OF POTASSIUM EXCREATED AT EACH INTERVAL WITH TIME

(TABLE - CXI)

	DOSE mg/kg		CONTROL	30 MIN	60 MIN	90 MIN	150 MIN	240 MIN	TOTAL AMOUNT OF POTASSIUM EXCREATED 4 HOURS AFTER CONTROL SAMPLE
VEHICLE	1 ml/100g		2.97	0.44	-0.44	0.88	-1.54	-0.69	-1.35
		E.O.V.S.	0	0	0	0	0	0	0
INDOMETHACIN	4		4.15	0.55	-2.98	0.07	-0.51	0.39	-2.48
		E.O.V.S.	1.18	0.11	-2.54	-0.81	1.03	1.06	-1.23
DEXAMETHASONE	4		1.72	0.66	0.32	-1.34	0.77	-0.34	0.07
		E.O.V.S.	-1.25	0.22	0.76	-2.68	2.31	0.35	1.42
HYDROCORTISONE	4		25.13	16.41	-16.41	-[illegible].69	2.54	-10.52	-18.21
		E.O.V.S.	22.16	15.97	-15.97	-8.57	4.08	-9.83	-16.86
HYDROCORTISONE	100		6.15	16.70	-16.48	-18.76	1.68	3.18	4.11
		E.O.V.S.	3.18	1.23	16.92	-19.64	3.22	3.87	5.46
FRUSEMIDE	4		40.0	-5.9	33.03	-46.41	13.37	-7.44	-20.77
		E.O.V.S.	37.03	-6.34	33.58	-47.29	14.91	-6.75	-19.42
FK10 IND	4		4.18	0.77	0.69	-0.51	0.82	2.85	2.05
		E.O.V.S.	1.21	0.33	1.13	-1.39	2.36	3.54	3.4
FK12 IND	4		3.08	5.97	-2.31	-2.12	0.85	-1.1	0
		E.O.V.S.	0.11	5.53	-1.87	-3.0	2.39	-0.41	1.35
FK12 AC	4		33.33	-2.56	-10.51	-8.29	1.11	-1.62	-27.26
		E.O.V.S.	30.36	-2.97	-10.07	-9.17	2.65	-0.93	-25.91
FK12 AC LACT.	4		7.18	1.87	-1.79	-1.72	5.64	-5.64	-4.41
		E.O.V.S.	4.21	1.43	-1.35	-2.6	7.18	-4.95	-3.06

E.O.V.S. - Effects of Vehicle Substracted

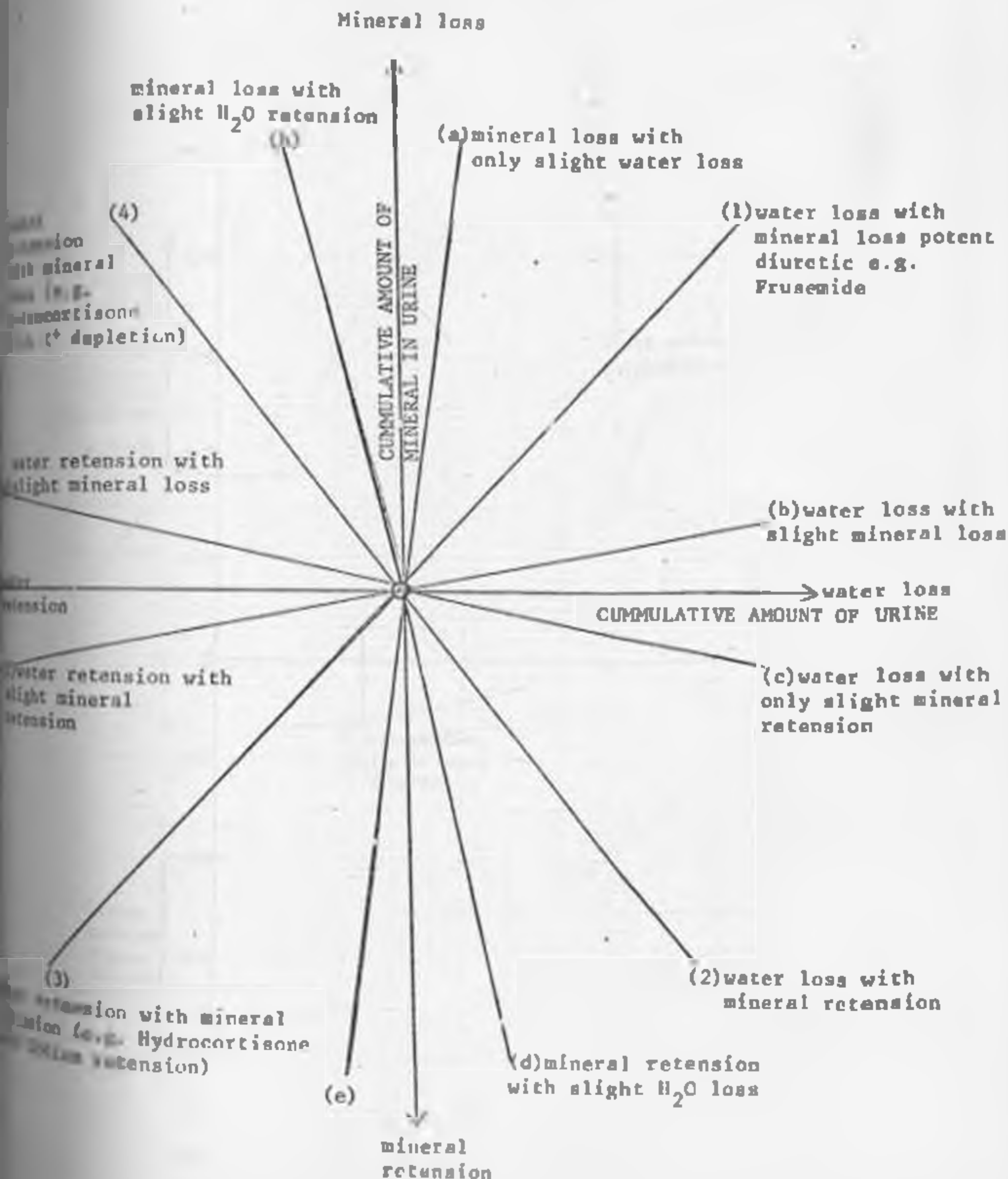
SUMMARY DATA ON RENAL ASPECTS: SIMULTENOUS CONSIDERATION OF WATER AND MINERAL EXCREATION - DATA FOR DOUBLE CUMMULATIVE PLOTS (TABLE - CXIII)

	CONTROL			30 MIN			60 MIN			90 MIN			150 MIN			240 MIN		
COMPOUND	CU	CS	CK	CU	CS	CK	CU	CS	CK	CU	CS	CK	CU	CS	CK	CU	CS	CK
VEHICLE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
INDOMETHACIN	3.5	2.25	1.18	2.5	-3.0	2.47	1.5	-5.7	1.22	4	-7.4	-0.84	5	-8.96	-1.87	6	-9.26	-1.82
DEXAMETHASONE	0	-2.26	-1.25	-1.0	-3.61	-2.28	-4.0	-3.83	-2.55	-6.0	-5.23	-5.04	-12.0	-2.6	-5.22	-17.0	-1.52	-5.1
HYDROCORTISONE	7	12.83	22.16	9.5	39.65	60.29	8.5	45.95	82.5	6.0	52.69	96.04	0.5	58.64	111.2	-6.5	62.21	116.5
FRUSEMIDE	5	19.76	37.03	11.5	29.65	67.72	1d	35.21	131.93	25.5	40.56	148.85	32.5	51.08	173.6	39	58.56	190.8
FK10 IND	7.5	-2.955	1.21	10.5	-4.74	2.75	12.5	-6.01	5.42	10.5	-6.23	6.7	4	-7.15	7.8	-3.5	-6.54	12.4
FK12 IND	6.5	-2.73	0.11	15	0.35	5.75	23.5	2.78	9.52	32	2.83	10.29	36.5	2.63	11.2	40.5	2.8	13.6
FK12 AC	5	37.18	30.36	7.5	61.4	57.72	5	82.6	75.01	8	92.6	83.47	6	99.21	89.2	4.5	105.04	94.0
FK12 AC LACT	8	4.695	4.21	14	9.87	9.85	21.5	11.65	14.14	25	12.39	15.83	25.5	13.21	21.9	27	13.43	23.1

CU - Cummulative amount of urine (mls)

CS - Cummulative amount of Sodium in urine)
) mEq per litre x 10^{-2}
CK - Cummulative amount of Potassium in urine)

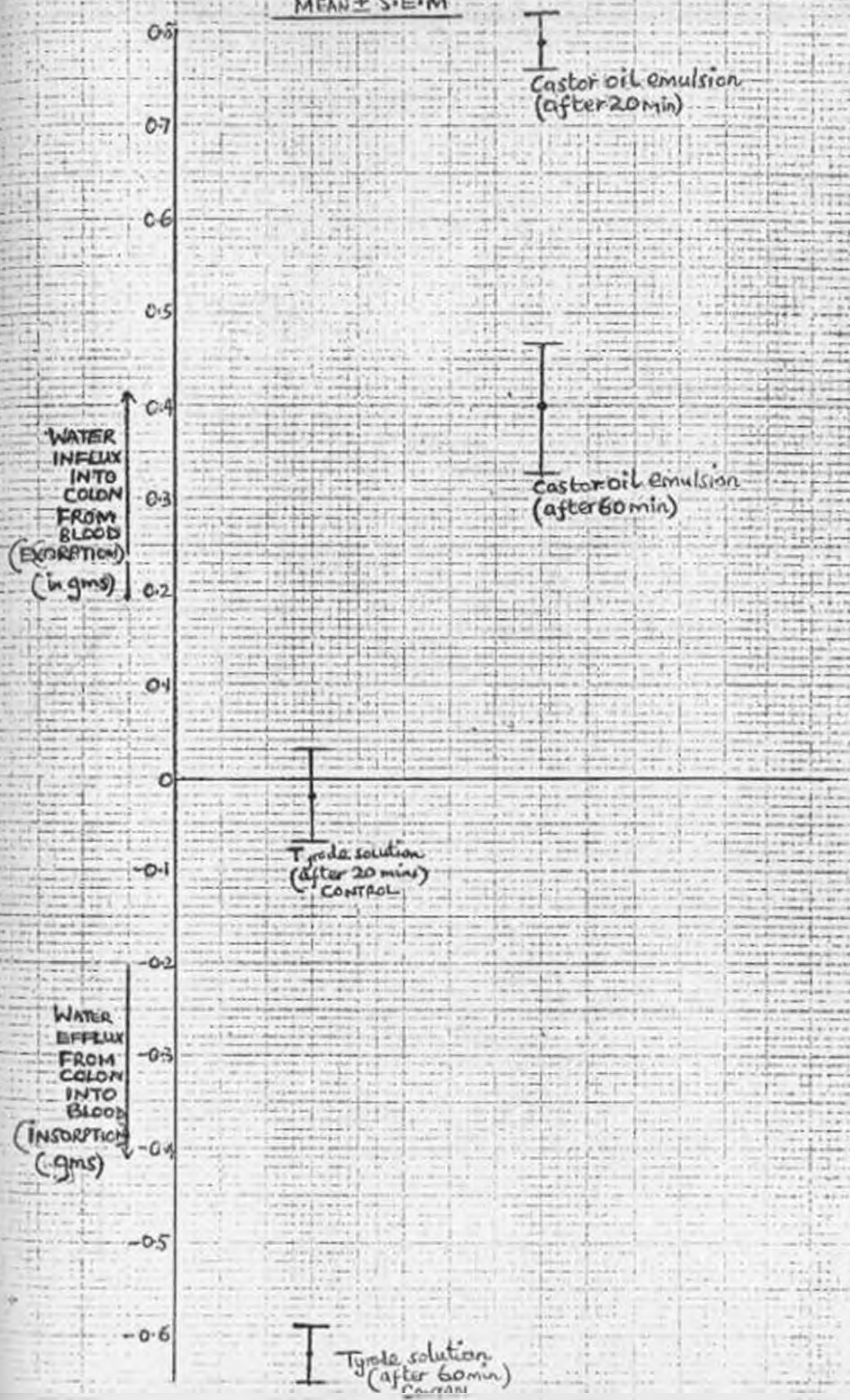
MODEL USED IN THE QUALITATIVE ASSESSMENT OF THE RENAL ASPECTS

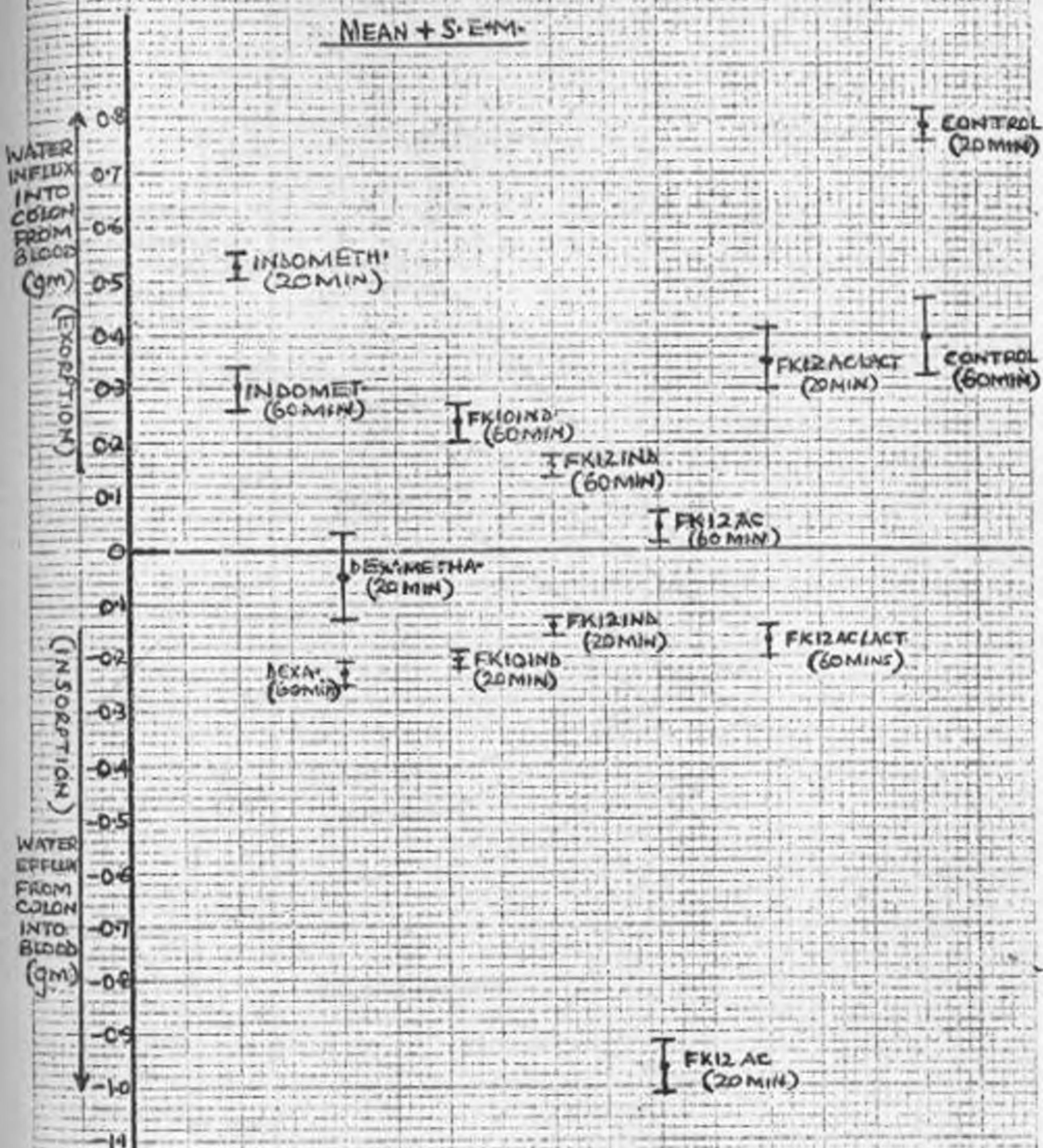


VEHICLE IS TAKEN AS THE REFERENCE POINT (O,O)

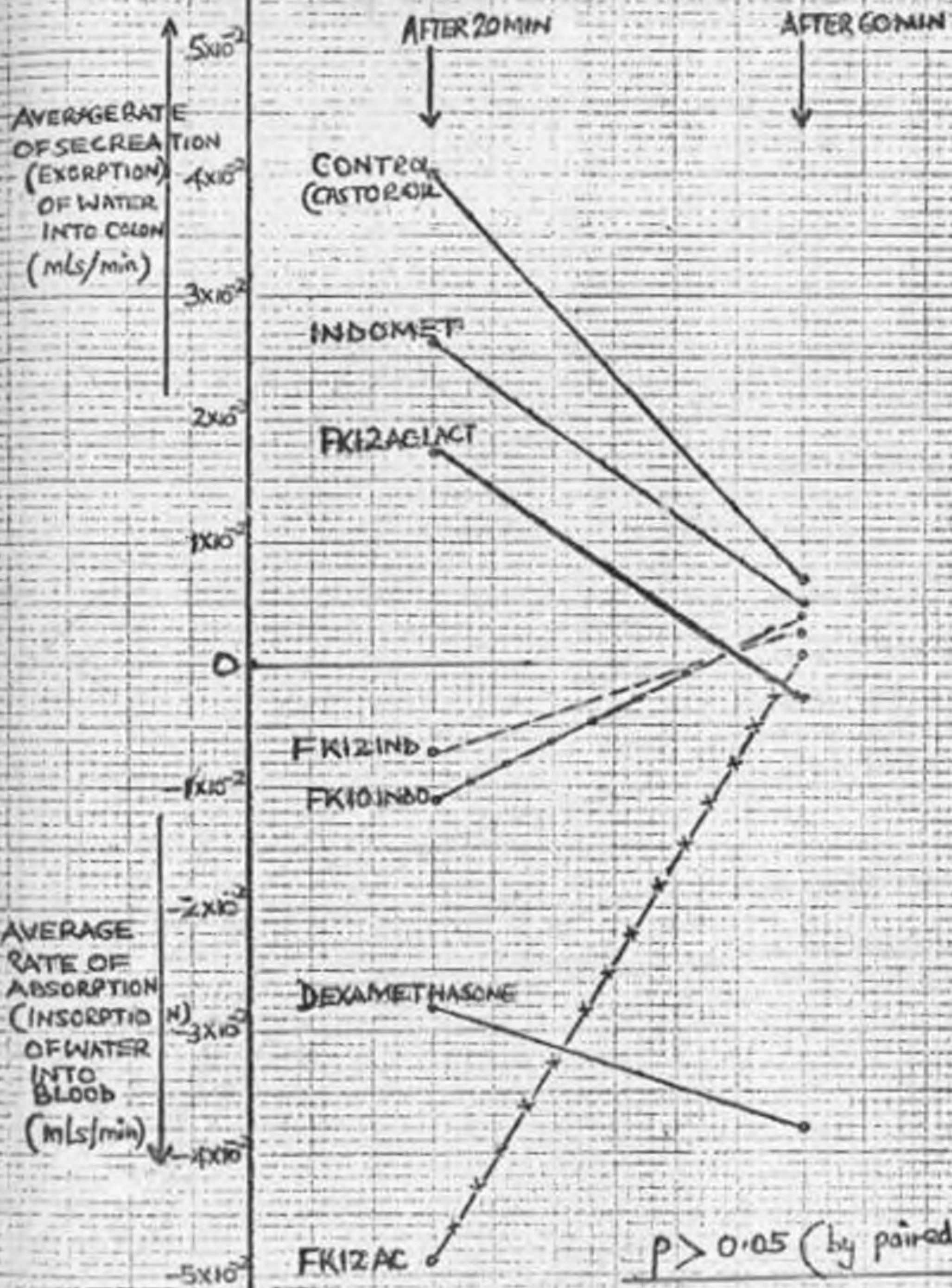
- Experimentally the effects at a & h, b & c, d & e, f & g micht be found to overlap (see interpretation).
- Obviously the magnitude of deviation along a certain path from the vehicle determines the significance of the response.

EFFECT OF CASTOR OIL ON COLONIC WATER FLUX (PLOT-BT)



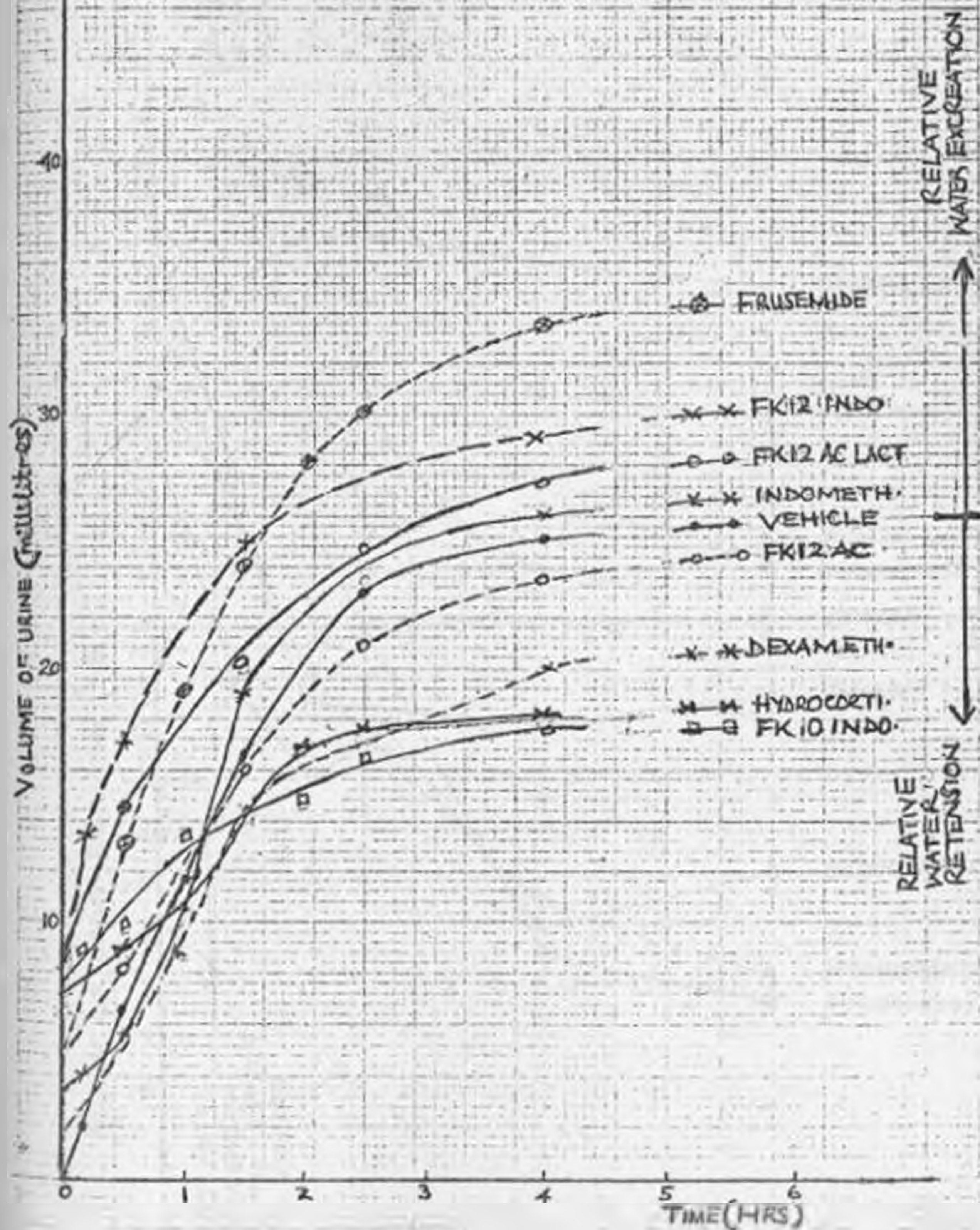

EFFECT OF PRETREATMENT WITH VARIOUS DRUGS ON CASTOR OIL-INDUCED COLONIC WATER-FLUX (PLOT.-BII)
MEAN + S.E.M.
WATER INFLUX INTO COLON FROM BLOOD (gm)
(EXORPTION)
(INSORPTION)
WATER EFFLUX FROM COLON INTO BLOOD (gm)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-0.9
-1.0
-1.1
CONTROL (20 MIN)
INDOMETH. (20 MIN)
FK12ACLACT (20 MIN)
CONTROL (60 MIN)
INDOMET. (60 MIN)
FK10IND (60 MIN)
FK12IND (60 MIN)
FK12AC (60 MIN)
DEXAMETHA. (20 MIN)
FK12IND (20 MIN)
FK12ACLACT (60 MINS)
FK10IND (20 MIN)
FK12 AC (20 MIN)

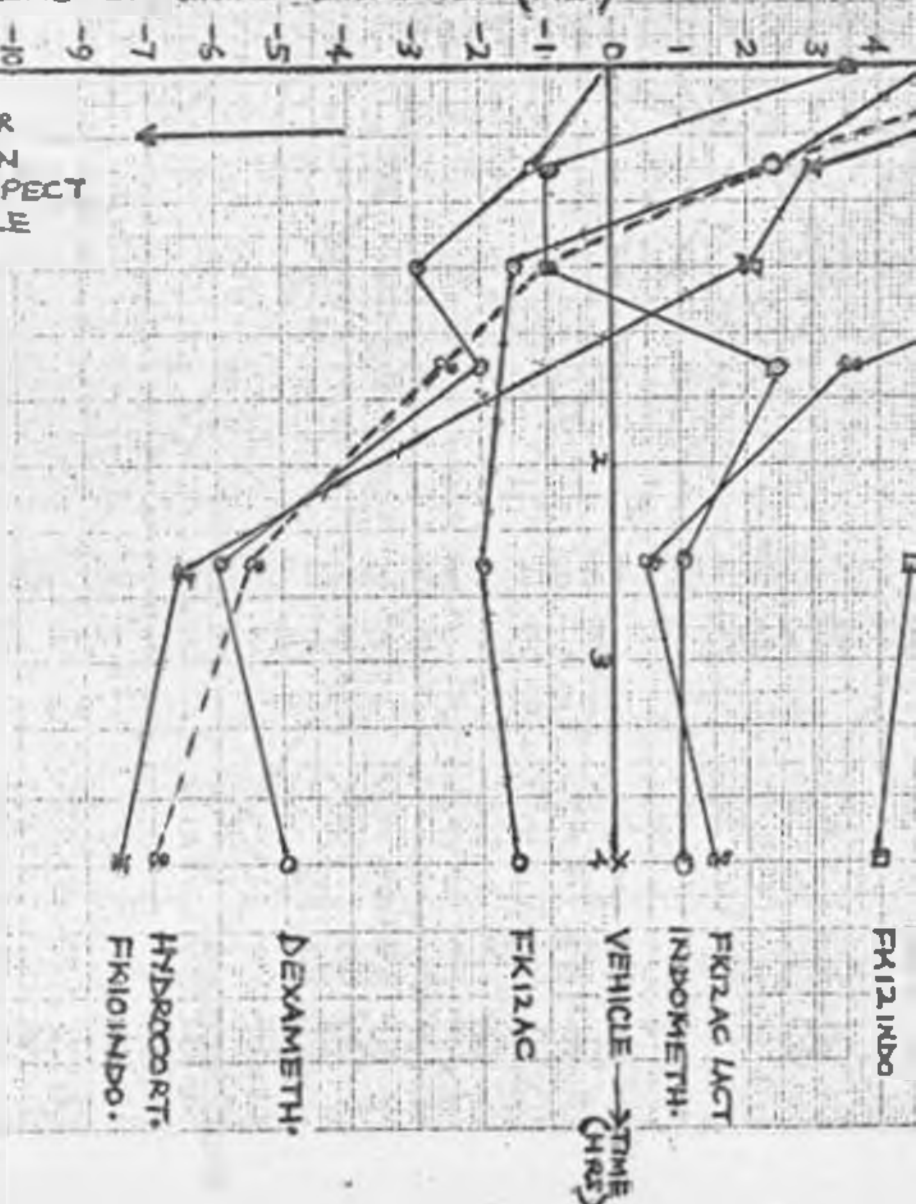
EFFECT OF PRETREATMENT WITH DRUGS ON RATE OF CASTOR OIL INDUCED COLONIC WATER-FLUX (PLOT — RIII)



EFFECT OF DRUGS ON WATER METABOLISM

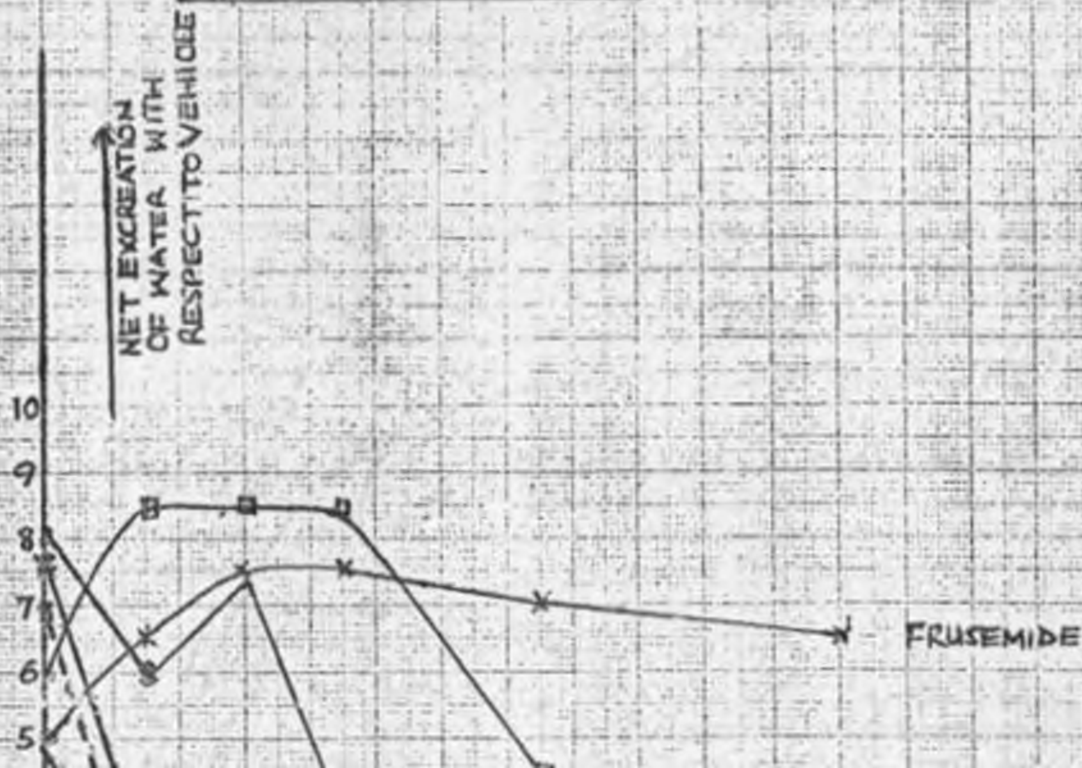
VOLUME OF URINE (mls) AGAINST TIME (HRS) (PLOT — CI)



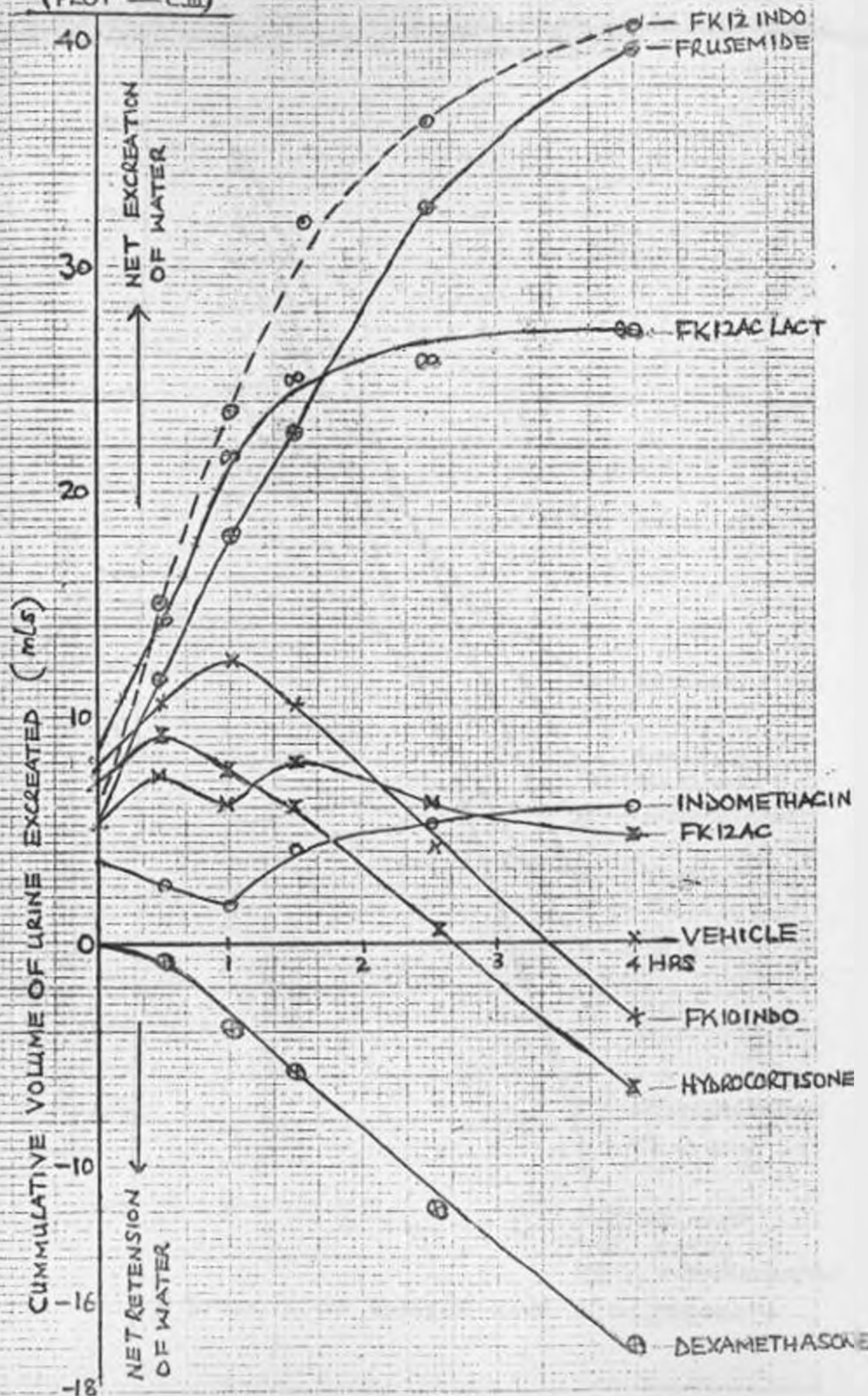


VOLUME OF URINE EXCREATED (mls)
-10 -9 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4
NET WATER RETENSION WITH RESPECT TO VEHICLE
1
3
4
FK101INDO.
HYDROCORT.
DEXAMETH.
FK12AC
VEHICLE
TIME (HRS)
INDOMETH.
FK12AC LACT
FK12INDO

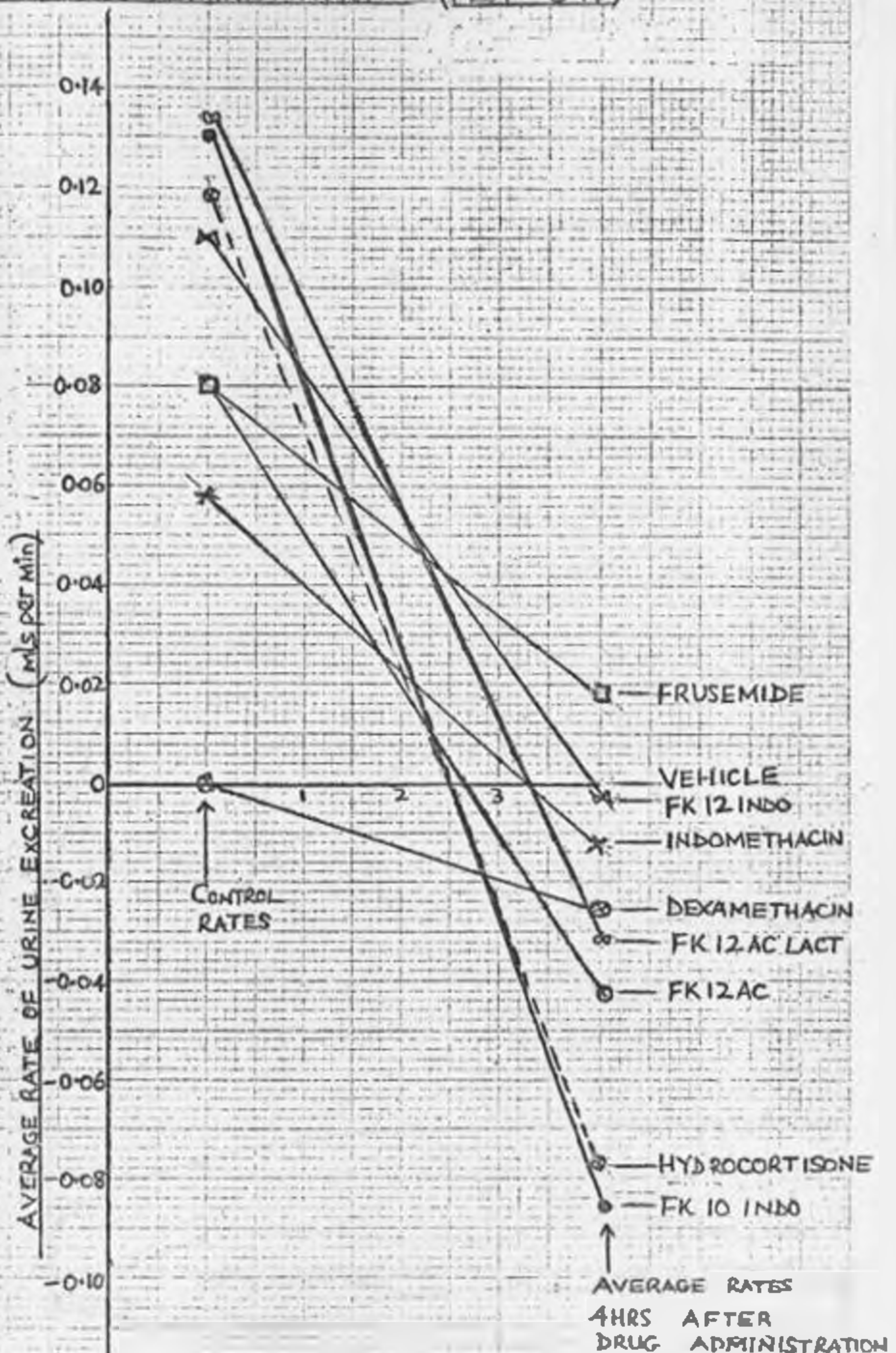
VOLUME OF URINE EXCREATED AGAINST TIME: (TAKING VEHICLE VOLUME AS BASELINE)



CUMMULATIVE AMOUNT OF URINE EXCREATED AGAINST TIME

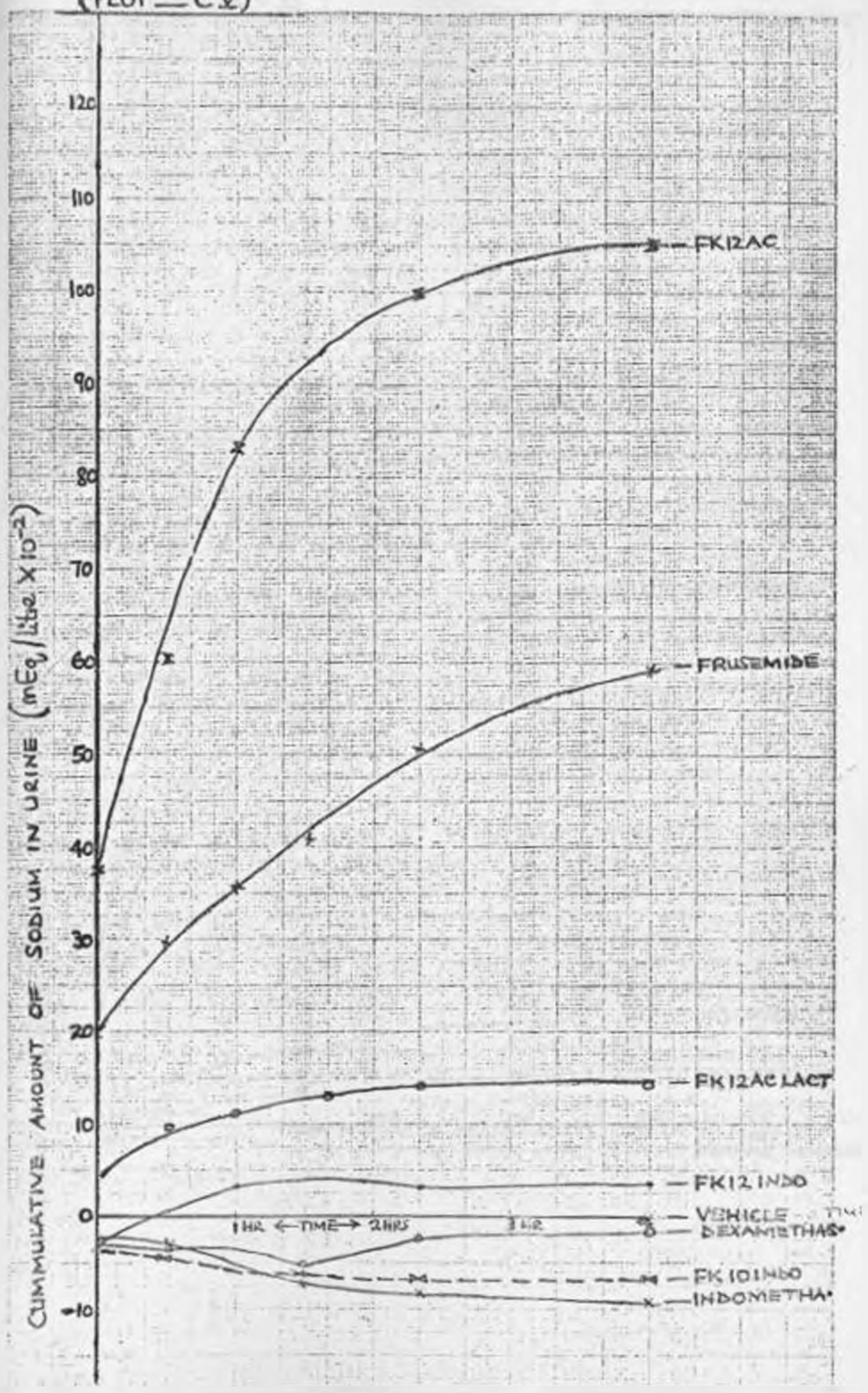


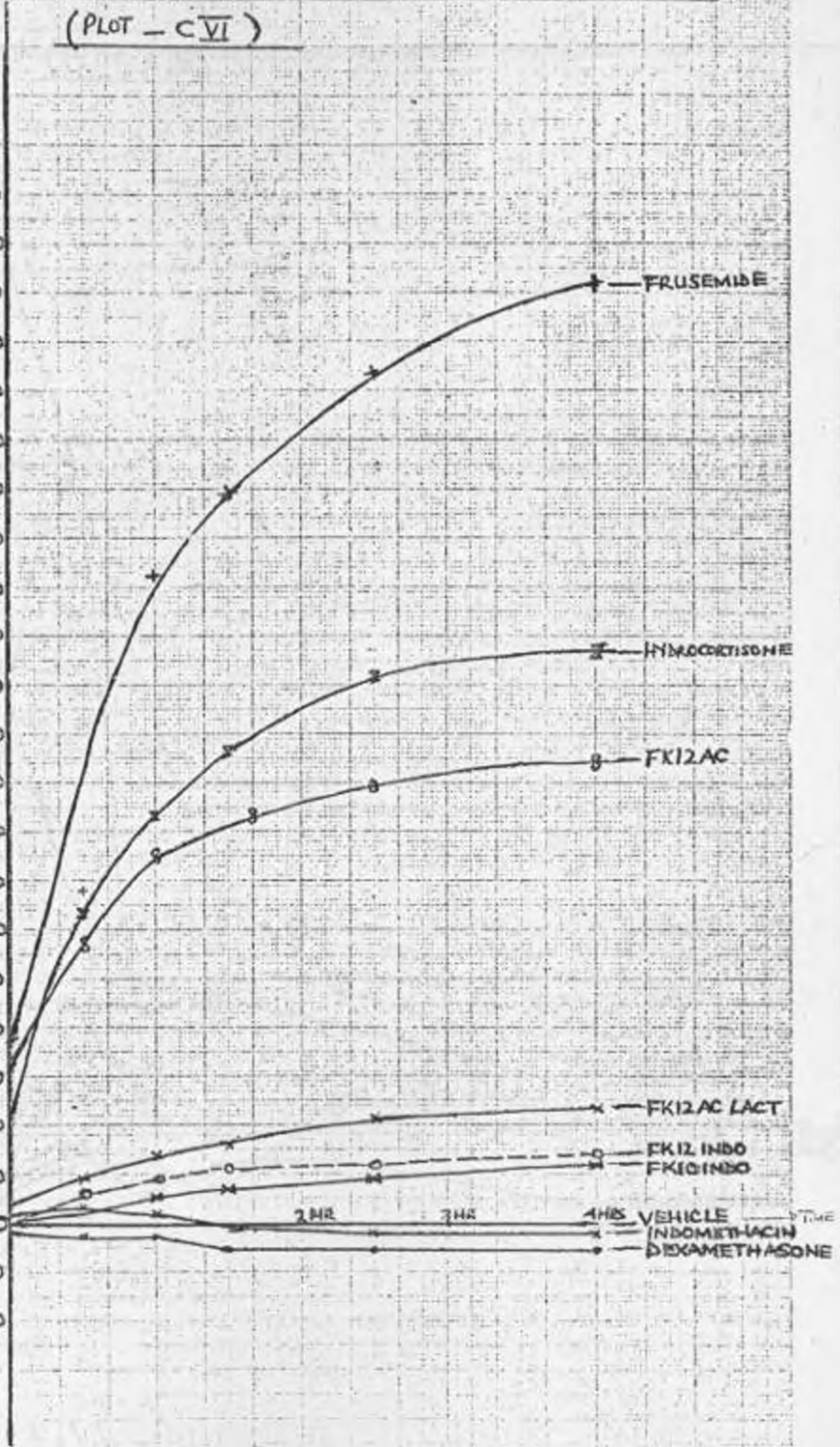
AVERAGE EXCREATION RATE OF URINE AGAINST TIME: COMPARISON BETWEEN CONTROL SAMPLE RATES AND AVERAGE RATES 4 HOURS AFTER DRUG ADMINISTRATION (PLOT C IV)



CUMMULATIVE AMOUNT OF SODIUM IN URINE AGAINST TIME:

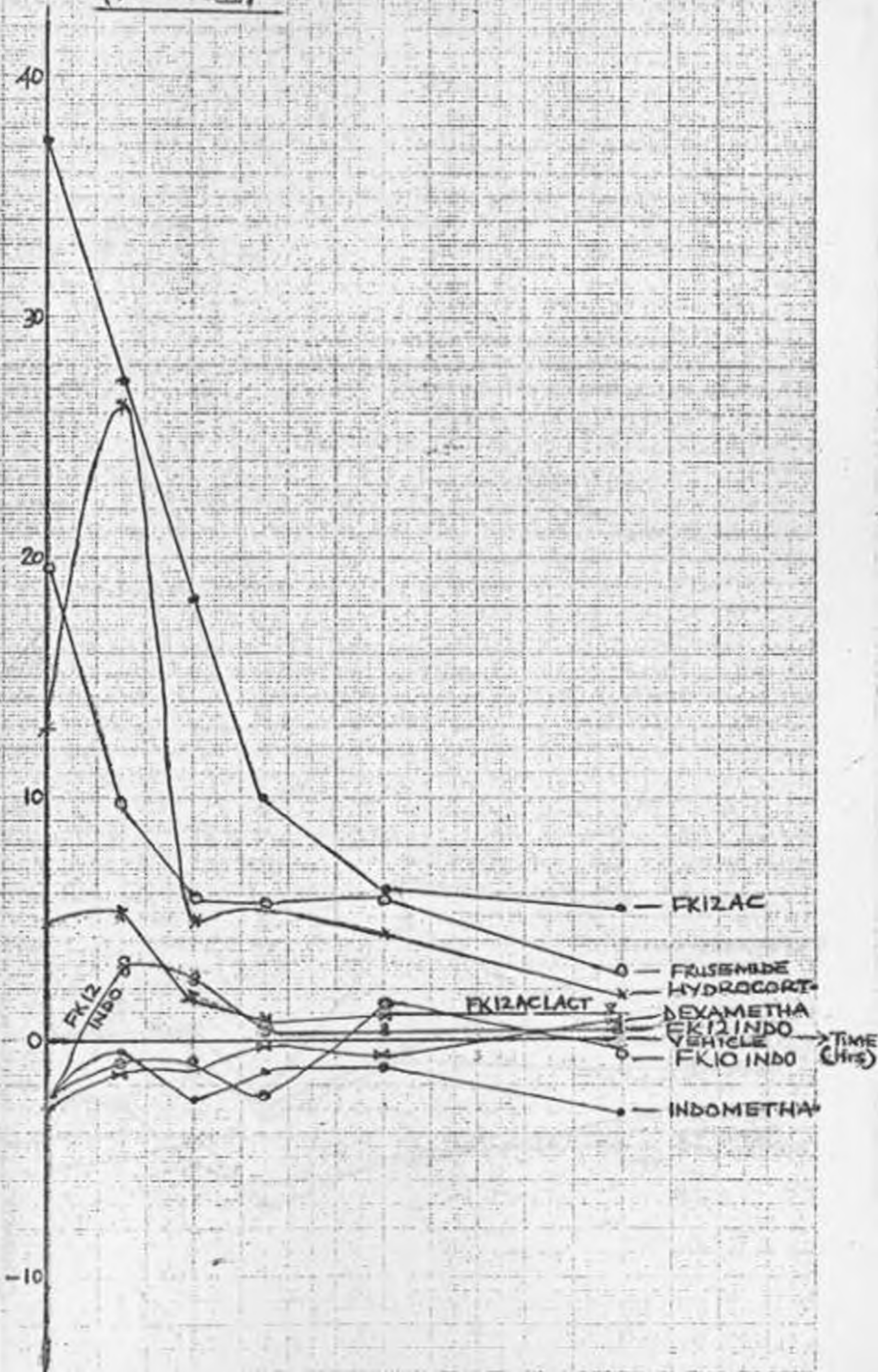
(PLOT — C V)




CUMMULATIVE AMOUNT OF POTASSIUM IN URINE AGAINST TIME :
(PLOT – C VI)
CUMMULATIVE AMOUNT OF POTASSIUM IN URINE (mEq/litre X10⁻²)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
2 HR
3 HR
4 HRS
TIME
FRUSEMIDE
HYDROCORTISONE
FK12AC
FK12AC LACT
FK12 INDO
FK10 INDO
VEHICLE
INDOMETHACIN
DEXAMETHASONE

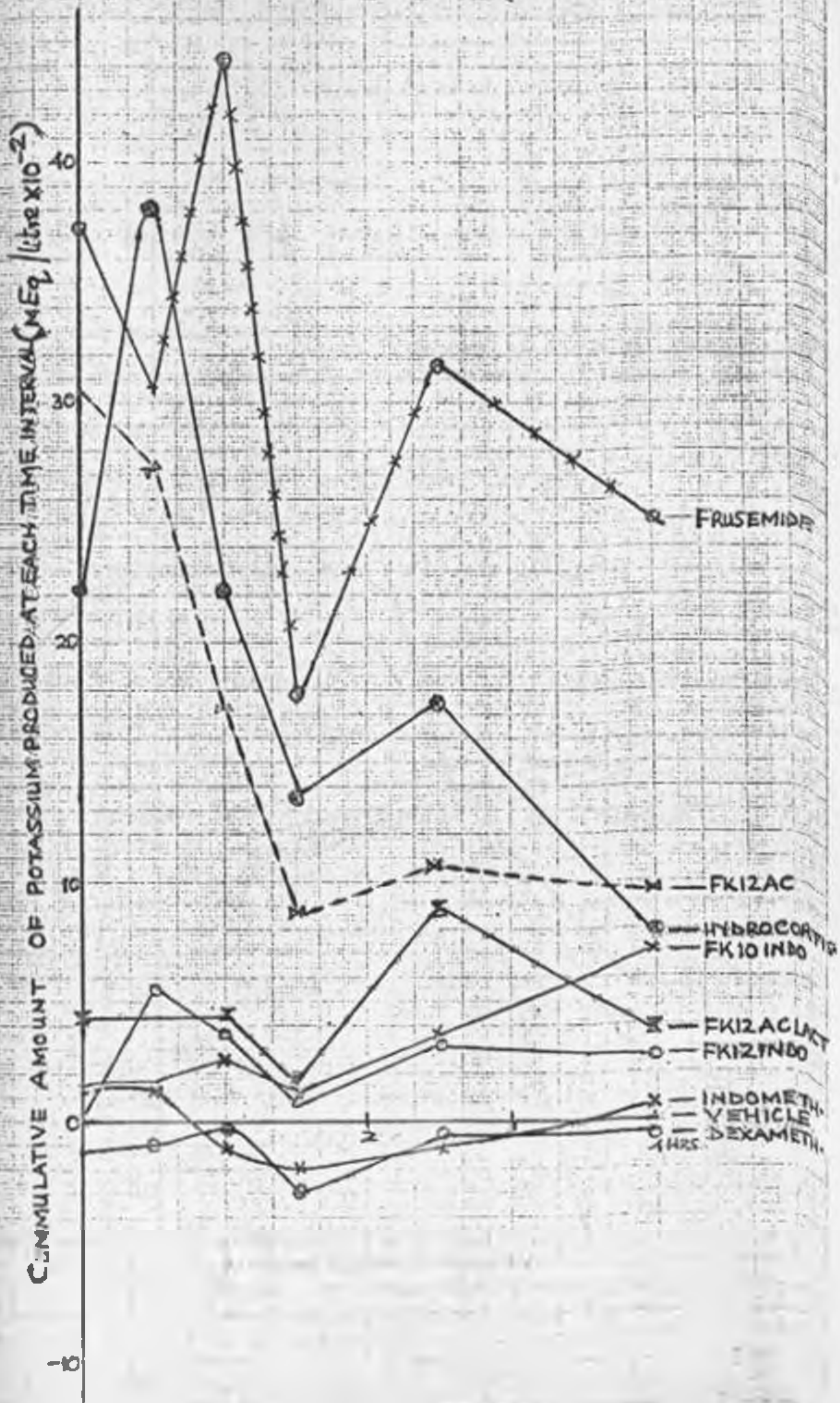
CUMMULATIVE AMOUNT OF SODIUM PRODUCED AT EACH TIME INTERVAL AGAINST TIME:

(PLOT-C VII)



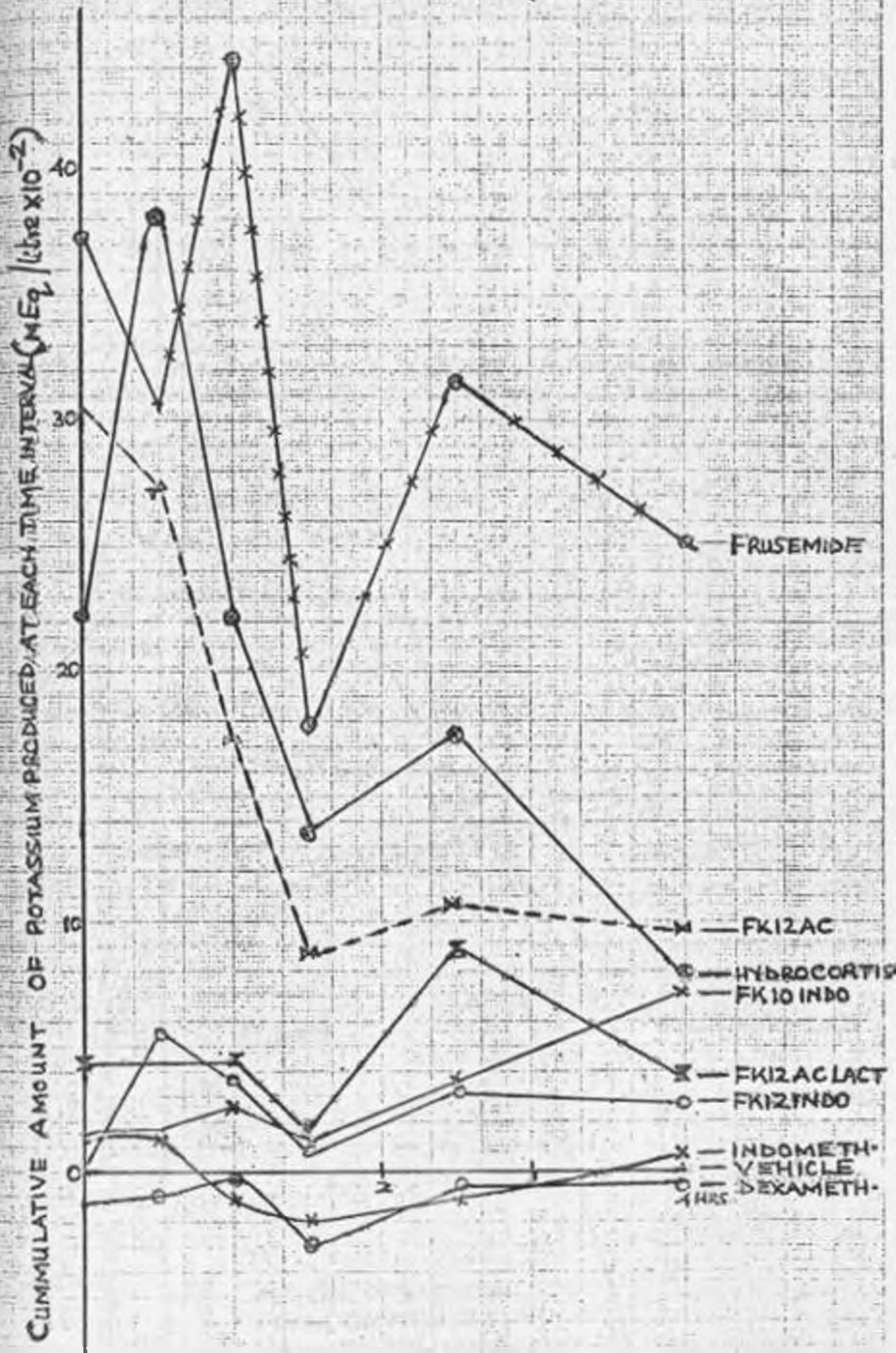
CUMMULATIVE AMOUNT OF POTASSIUM PRODUCED AT EACH TIME INTERVAL AGAINST TIME:

(PLOT — C VIII)

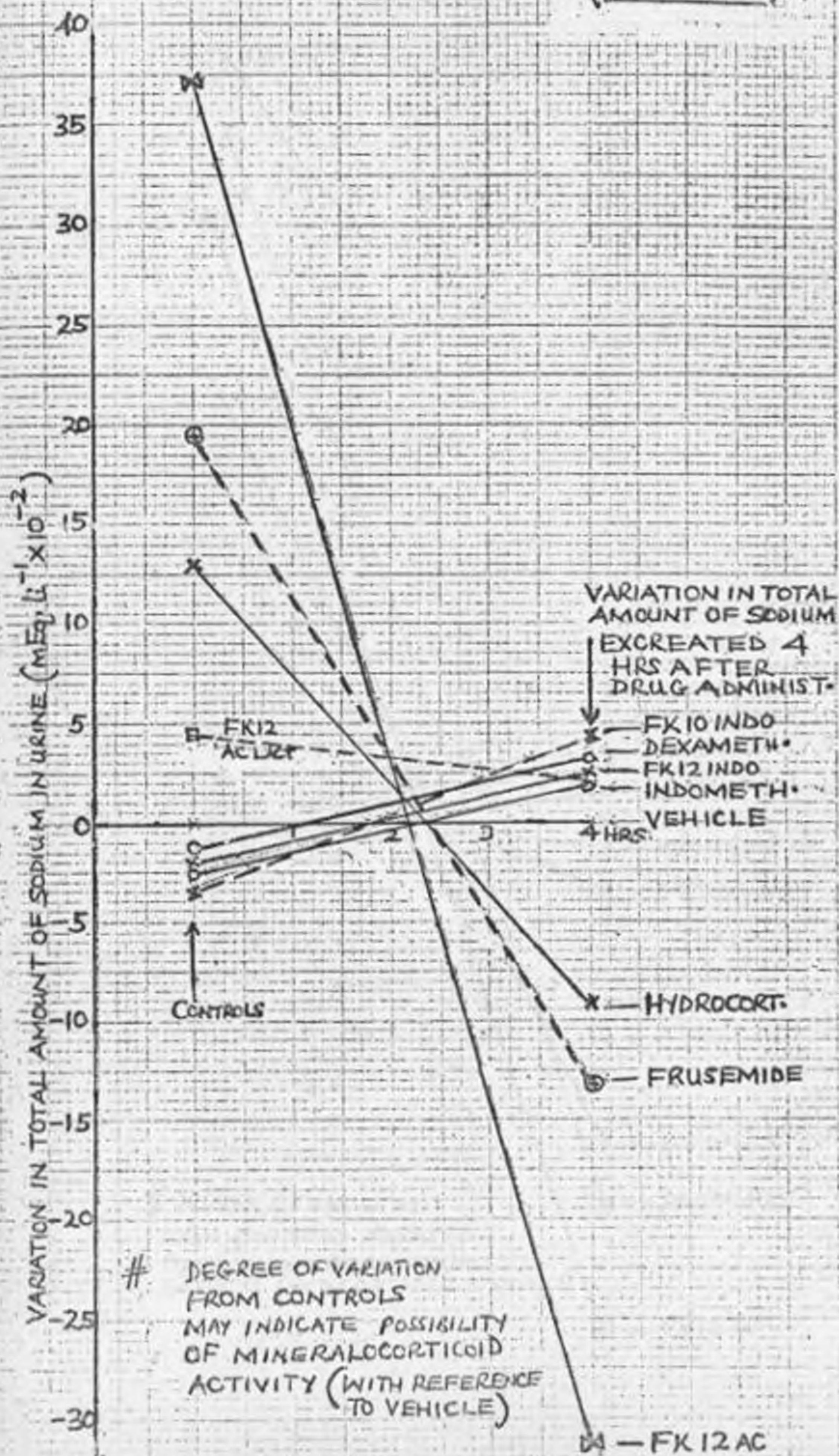


CUMMULATIVE AMOUNT OF POTASSIUM PRODUCED AT EACH TIME INTERVAL AGAINST TIME:

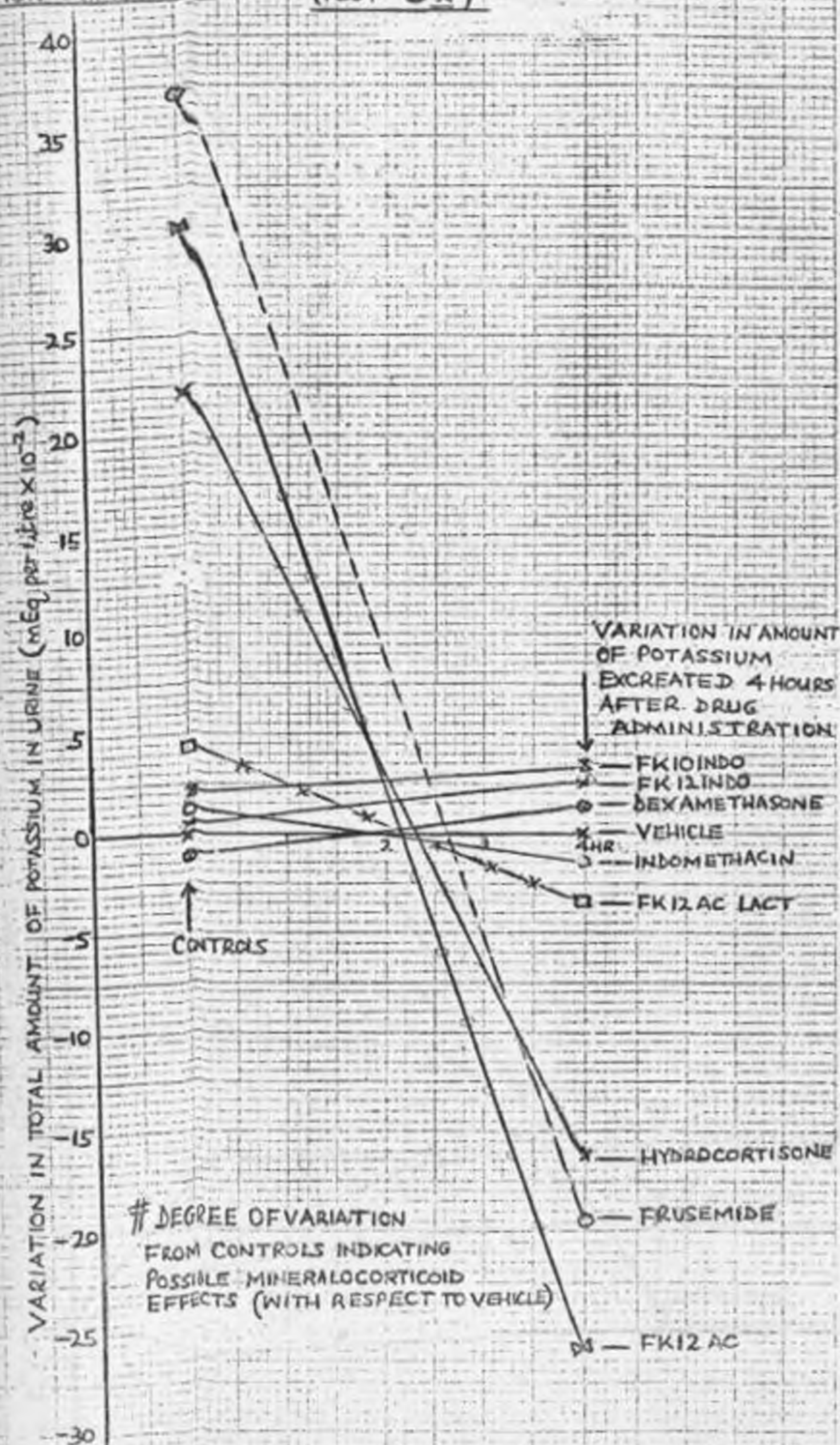
(PLOT — C VIII)

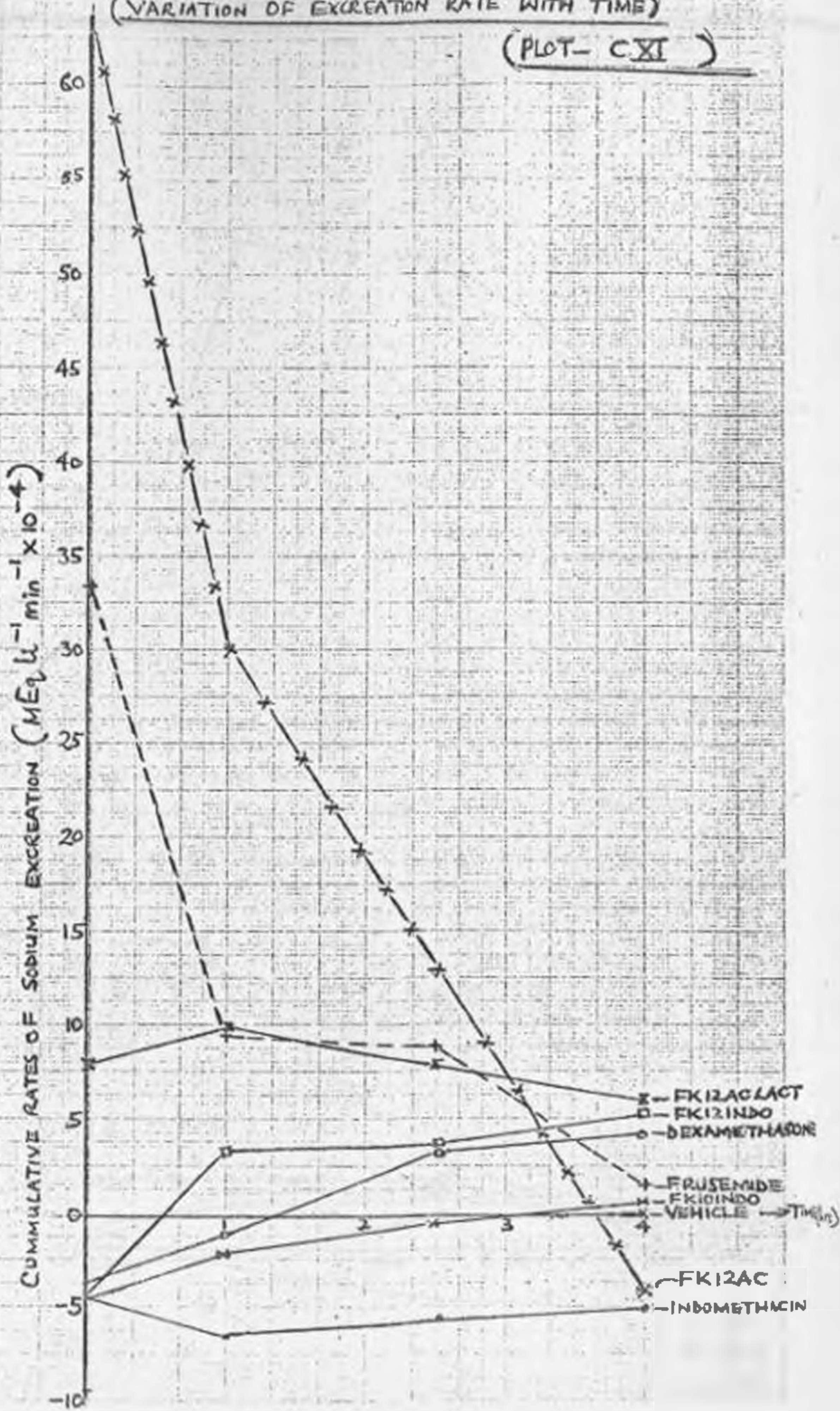


VARIATION IN SODIUM EXCREATION WITH TIME: COMPARING EXTENT OF VARIATION BETWEEN CONTROL SAMPLES AND 4HR-DURATION SAMPLES AFTER DRUG ADMINISTRATION (PLOT — IX)



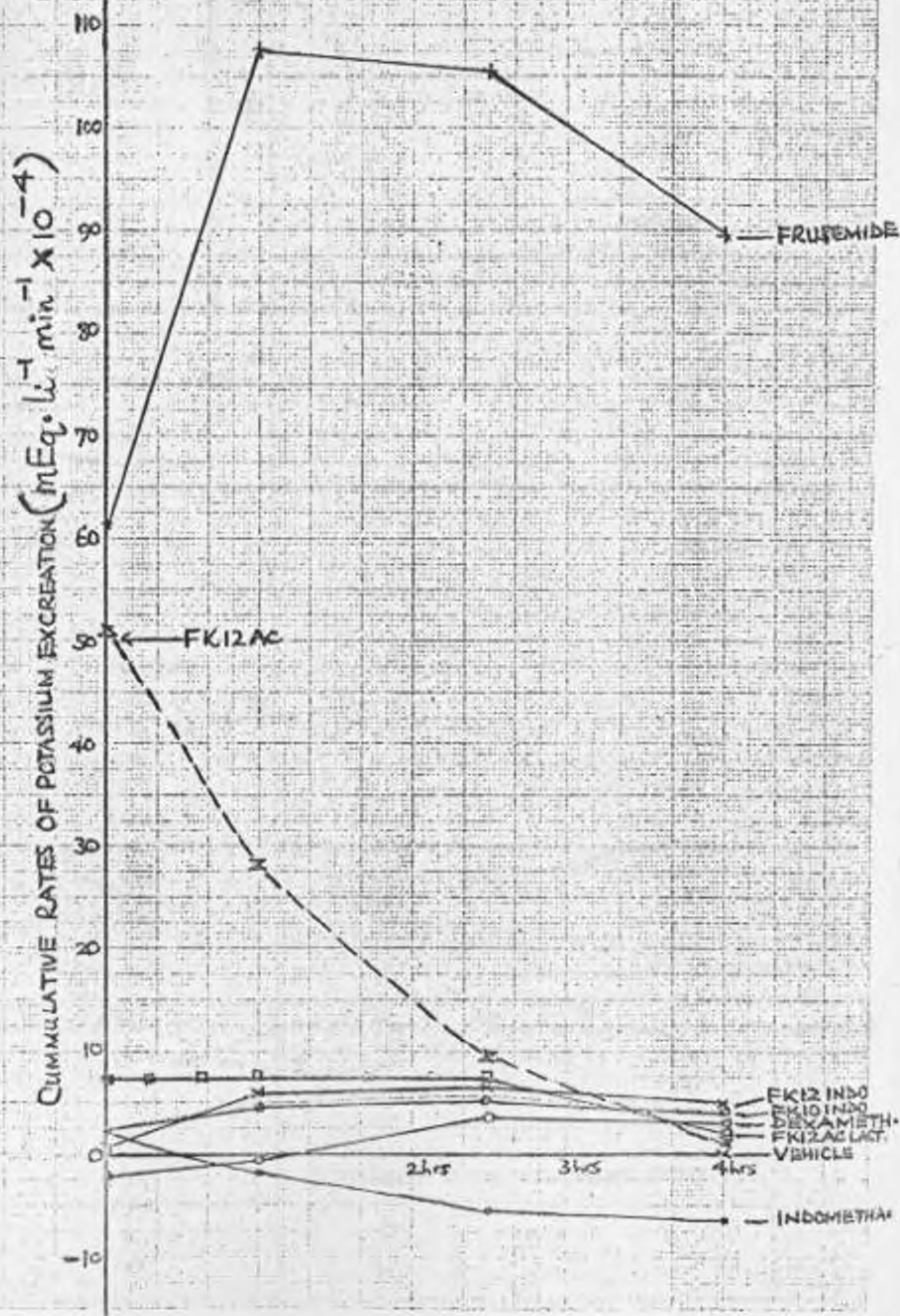
VARIATION IN POTASSIUM EXCREATION WITH TIME: COMPARING EXTENT OF VARIATION BETWEEN CONTROL SAMPLE AND 4 HOUR DURATION SAMPLES (PLOT CX)

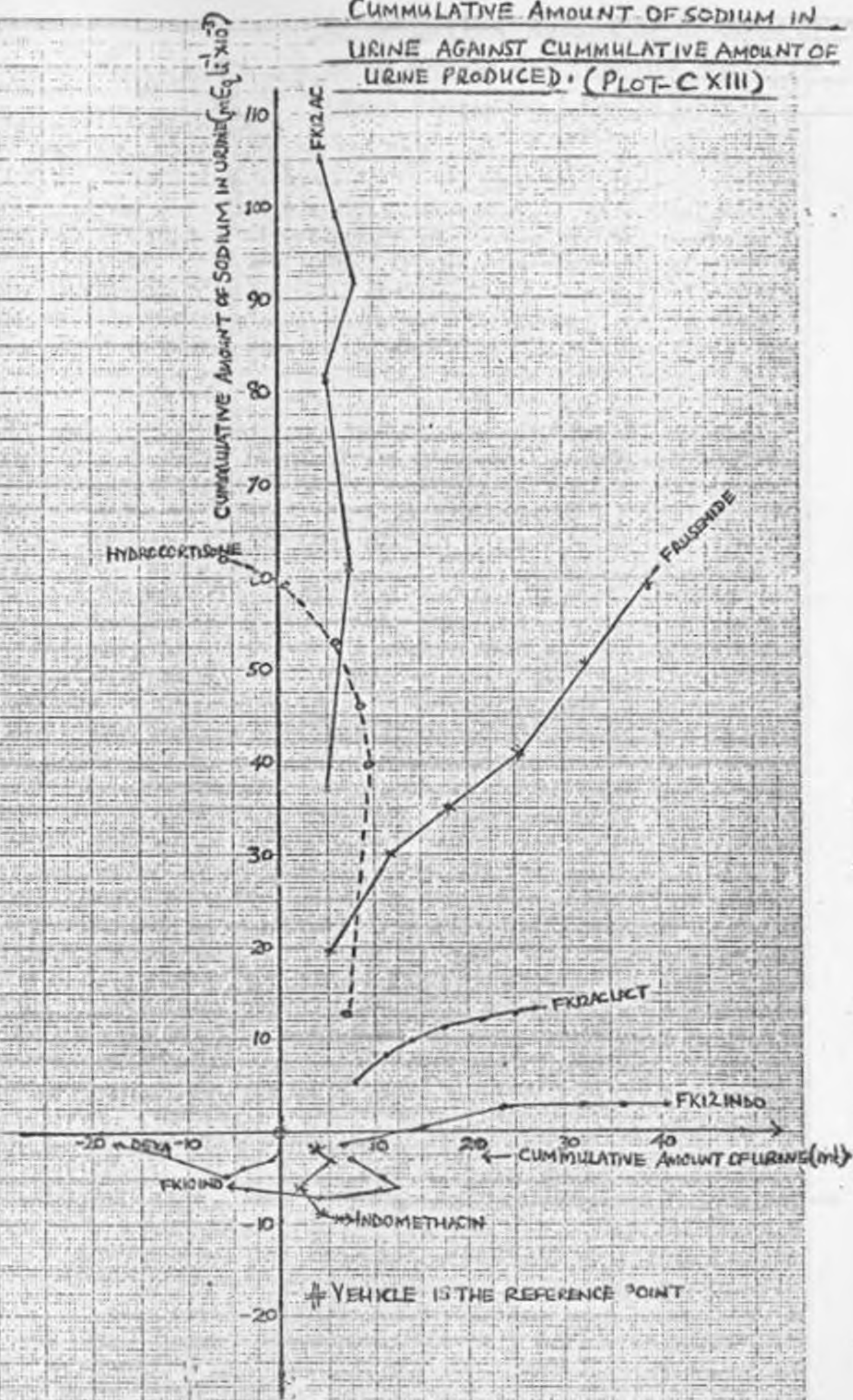


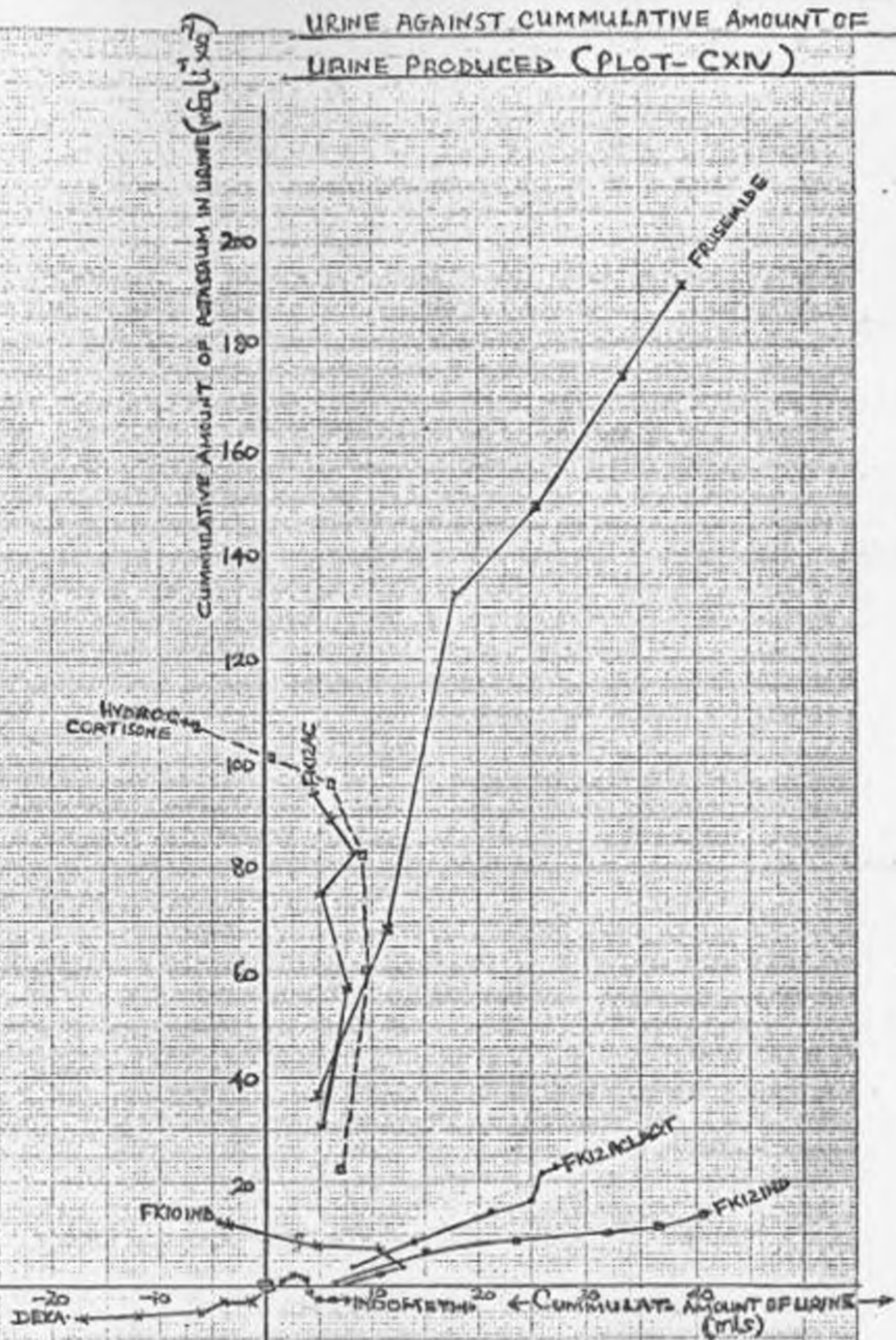

CUMMULATIVE RATE OF SODIUM EXCREATION AGAINST TIME:
(VARIATION OF EXCREATION RATE WITH TIME)
(PLOT- CXI)
CUMMULATIVE RATES OF SODIUM EXCREATION (MEq U⁻¹ min⁻¹ x 10⁻⁴)
-10
-5
0
5
10
15
20
25
30
35
40
45
50
55
60
1
2
3
4
TIME (m)
FK12ACLACT
FK12INDO
DEXAMETHASON
FRUSEMIDE
FK10INDO
VEHICLE
FK12AC
INDOMETHICIN

CUMMULATIVE RATE OF POTASSIUM EXCREATION AGAINST TIME: (VARIATION OF EXCREATION RATE WITH TIME)

(PLOT — C XII)




CUMMULATIVE AMOUNT OF SODIUM IN URINE AGAINST CUMMULATIVE AMOUNT OF URINE PRODUCED. (PLOT-C XIII)
CUMMULATIVE AMOUNT OF SODIUM IN URINE
FK12AC
HYDROCORTISONE
FRUSEMIDE
FK12ACUCT
FK12INDO
DEXA
FK10IND
INDOMETHACIN
CUMMULATIVE AMOUNT OF URINE (ml)
VEHICLE IS THE REFERENCE POINT



CUMMULATIVE AMOUNT OF POTASSIUM IN URINE AGAINST CUMMULATIVE AMOUNT OF URINE PRODUCED (PLOT- CXIV)
CUMMULATIVE AMOUNT OF POTASSIUM IN URINE
200
180
160
140
120
100
80
60
40
20
-50
FRUSEMIDE
HYDROCORTISONE
FK12AC
FK12ACLACT
FK12IND
FK10IND
INDOMETH
DEXA
-20
-10
10
20
30
40
CUMMULATIVE AMOUNT OF URINE (mls)
VEHICLE IS THE REFERENCE POINT (0,0)

D I S C U S S I O N

DISCUSSION

Inhibition or delay in castor oil-induced diarrhoea has become a fashionable model in various laboratories for assessing PG Synthetase Inhibitors. This model is tailor made to suit any compound whose mechanism of action is at lease mediated via blockade of the Cyclo-oxygenase enzyme. In this model potent PG-biosynthesis inhibitors block cyclo-oxygenase and this is attended by a decrease in level of prostaglandins from the Arachidonic acid cascade. That Aspirin like drugs act by inhibiting Cyclo-oxygenase was discovered by Vane et al in 1971 (5) and that in 1978 Awounters et al (142) found that inhibition or delay in castor oil induced diarrhoea is a response which accompanies pre-treatment of rats with a putative anti-inflammatory agent. By varying the doses Awounters et al found a relationship between degree of inhibition and potency of the compound. Further they found that doses needed for complete inhibition of the diarrhoea fell rapidly within the toxic range (hence agreeing with the fact that therapeutic use for Aspirin-like drugs against diarrhoea is not yet established).

The importance of this model in the search of new anti-inflammatory agents is two fold: namely - that it can be used to screen PG biosynthesis inhibitors (Aspirin like drugs) and that by varying the doses one can obtain ED_{50} and other parameters which help assess potency. The reproducibility of the results depends on the diarrhoeal evacuations which are characteristic, the onset of this quantal response indicating the time when no more protection can be afforded. That this model is useful was confirmed by these workers by considering the ED_{50} values obtained by this method relative to the Carageenan Rat paw oedema results of which showed remarkable concordance.

The results obtained in this work using the castor oil Test qualitatively indicated the 4 Novel synthetic compounds studied do in fact inhibit Cyclo-oxygenase and in so doing they delay and at times inhibit onset of diarrhoea. The potency of these compounds was not assessed as only 2-dose-level were used (4 mg and 10 mg per kg). It is suggested that more meaningful data on potency using this model can be obtained if:

(i) The number of animals used per dose can be increased (say from 6 to 50).

(ii) The number of dose levels be increased from low doses to very high doses (Awounters used doses ranging from 0.3 mg to 320 mg/kg). In doing this, one is obviously able to find ED_{50} of every drug that is a potential anti-inflammatory agent.

The results obtained with the colonic water flux experiments were primarily aimed at confirmed the results obtained with the castor oil test. The test is also based on ability of castor oil to increase P GE synthesis in the intestines (4 to 20). By increasing PGE synthesis in the intestines castor oil reverses the water absorption into blood stream into a net water secreation into colon (exorption). This method can be used qualitatively to assess the mode of action of putative NSAID since it is known that by inhibiting the cyclo-oxygenase level of PGs would be decreased and instead of observed water secreation into colon, the secretory effect of castor oil would be decreased and at times reversed into absorption obviously on the potency of the putative NSAID. The results obtained with these model helped to confirm those obtained by castor oil test in that all the FK compounds studied decreased the secretory effect. The extent of decrease in the secretory effect reflected the potency the compound as anti-inflammatory agent.

Having shown that the 4 FK compounds studied so far displayed same anti-inflammatory effect probably mediated via prostaglandin synthesis blockade their renal side effects on water and mineral excreation was studied and as a result have indicated FK12 AC has mineralocorticoid activity which is not accompanied by concomitant water loss and hence its use as anti-inflammatory in future being limited by side effects.

FK12 AC LACT shows anti-inflammatory effects but its potential diuretic effects (causes mineral and water loss) needs further investigation.

The fact that the FK12 INDO shows anti-inflammatory activity coupled with water excreation which is not accompanied by mineral loss poses yet another pu-zle:- could this compound be useful in clearing the oedema which usually accompanies some forms of inflammation? Could this compound be useful in inducing diuresis in which case it could be useful since it would not require mineral supplementation as it has no mineralocorticoid effects?

FK10 IND, the fact that it causes very little retension of water (less than dexamethasone) and that its mineralocorticoid effects are minor and further that its trend (Plot C13) resembles that of dexamethasone implies that this compound requires optimization. Obviously before any conclusions such as I have hazarded should be made, intensive data on renal aspects need to be accrued.

What these results imply is that a lot of work is still required to put the FK compounds in their proper perspective. Whereas this work and that done concurrently (153, 154, 159) indicate that the FK compounds have anti-inflammatory activity, the actual potency needs to be confirmed by more sensitive methods (141, 137, 118, 142, 128) using more animals. The actual mechanism of their action urgently needs to be confirmed using direct tests. As a suggestion the PG synthetase assay test elaborated by Vane et al (1971) should be done. The activity of radial labelled Arachidonic acid on sheep seminal vessicle (SSV) should provide a direct assay on the extent of Dehydrogenase blockade. Since the compound still posses some features of their steroidal counterparts the fact that they do not affect Phospholipase A_2 (of if they do) should directly be established (136).

As was cited earlier on all the known NSAID through chemically dissimilar share the same side effects namely: gastrointestinal irritation (ulceration potential), nephrotoxicity (reduction in renal blood flow, papillary necrosis), prolongation in parturation and pregnancy, inhibition platelet aggregation. There is thus an urgent need to find the extent of these side effects with FK compounds.

In studying gastrointestinal toxicity indomethacin induced ulcers are used to the extent of ulceration using the ulcer index and histological studies. Recently Japanese workers - Nakamura et al (1983) were able to come up with a non invasive model of measuring gastrotoxicity. The model is simple, acute and uses Phenol red as a marker. They were able using this model to show that Phenol red is absorbed in ulcerated animals and its appearance in urine was recorded spectrophotometrically (in fact this method can simultaneously be used to assess renal integrity) 157.

The acute need necessary to perform renal studies on FK compounds is based on the observation that anti-inflammatory analgesics cause

varying degrees of nephrotoxicity with some incidence, in high doses, of papillary necrosis. Some like Phenylbutazone cause retention of Sodium Chloride and water. Evidence exists that the renal toxicity of the compounds can be accounted for by inhibition of PG biosynthesis. It is now known that blood flow in the kidney is sustained by PG production in the medulla (37). There is continous outflow decreases (Aiken and Vane 1973). Naura and Chirawong 1974 in their studies on medullary blood flow in rats treated with NSAID showed the presence of Ischaemia which correlated well with the capacity of urine to concentrate and conserve sodium. What this indicates is that both chronic and acute data on FK compounds pertaining to renal side effects should be well assessed if they have to be of any therapeutic use. The present work has shed some light into the possible side effects with some of the compounds and the need of more data to support or refute is urgently needed.

Data on inhibition of platelet aggregation should be amassed as this might point out the future therapeutic use of the agents in thrombosis (36). Studies on possible roles of PGs on uterine motility have been done (Williams & Vane 1975, 69, 45, 46, 36, 43). From these studies it does appear that PG synthesis inhibitors can be used in relieving pain due to dysmennorrhoea, the fact that NSAID have been tried for this purpose (114, 115, 116) calls for a need to perform similar studies on effects of FK compoundson reproductive system (prolongation of pregnancy and parturation).

In conclusion what can be said is that a lot remains to be established with FK compounds. However, the little that has been established suggests that these compounds have a high anti-inflammatory activity i.e. are very potent as is borne out by this present work using the castor oil test and confirmed by the colonic water influx experiment. Other workers also high potency of the compounds (153, 154, 159). The pKa, Log. P values have been established and found to be suggesting of very potent compounds (153). General SAR indicate them to be neither steroidal nor convection non-steroidal, they are compounds which in essence bridge the gap between the two groups. Some are acids while the others are lactams and indeed what does emerge from their structure is that they are lipophilic.

It cannot be over emphasized that enhanced lipophilicity is a neccessary prerequisite since therapeutic usefulness of any anti-inflammatory agent depends on its ability to distribute into the inflammed areas. Detailed SAR as to account for their effects has not been fully established and the urgent need to perform studies on the side effects cannot be over emphasized.

Finally, the fact that activity with FK compounds has been screened and potency suggested to be high and with attempts on side effects studies started, the stage is now set for pharmacokinetic studies to yield data for the future therapeutic use of this novel-synthetic, non-ubiquitous and fascinating compounds.

P L A T E S

PLATE 1

Inhibition of Castor Oil-induced Colonic Water Flux:

Below is a photograph showing two rats dissected under anaesthesia to expose the colon. The rat on the left had its colon ligated and instilled with the vehicle. The rat on the right had its colon instilled with castor oil after ligation.

It is observed that the colon of the rat on the right is more distended due to water secreation into colon from circulation. FK compounds, vehicle, indomethacin, dexamethasone can be seen in the background (see text).

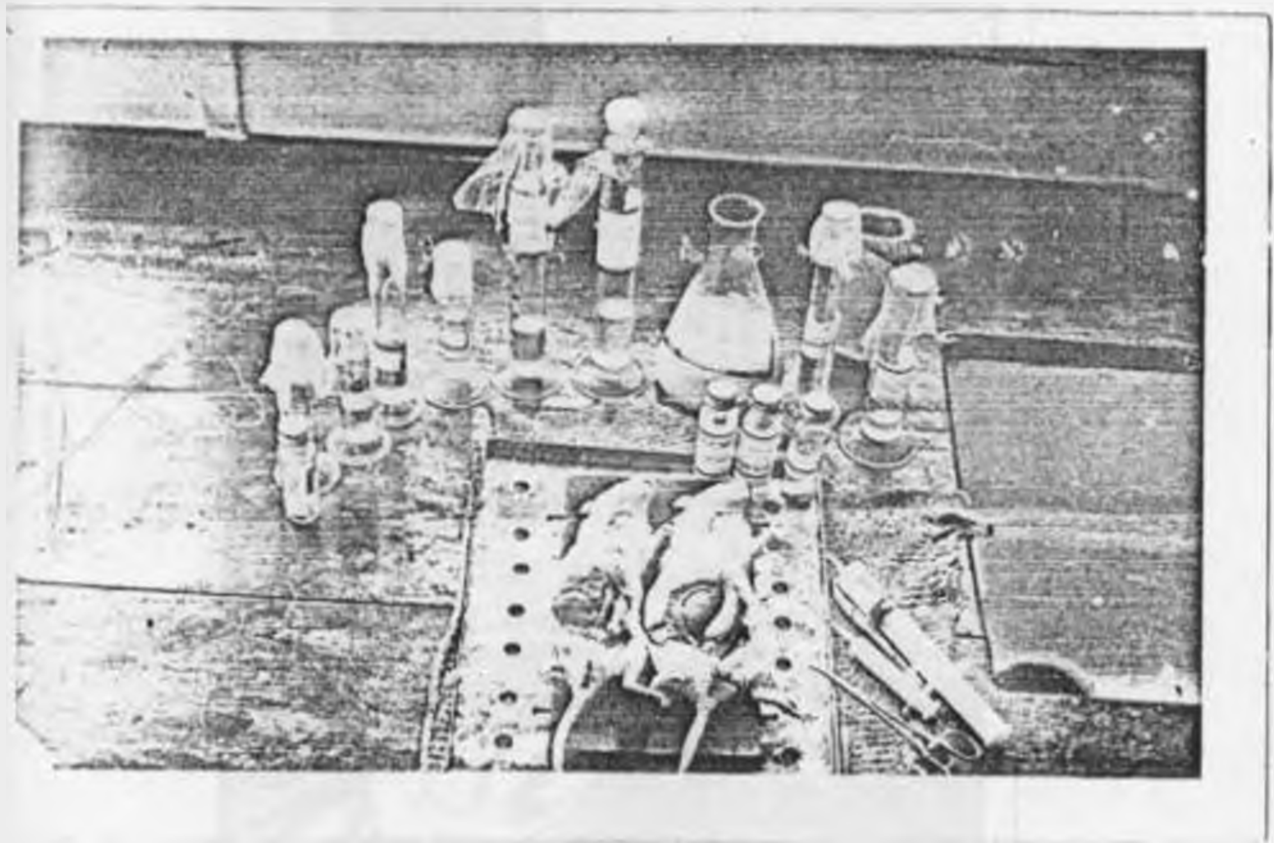


PLATE 2

Inhibition of Castor Oil Colonic Water Flux:

Below is a photograph showing two sections of the colon, isolated and placed in the petrish. The section of colon on the left (pointed by the author) was isolated from the rat that was treated with castor oil (plate 1) and the colon - section on the right was isolated from the rat treated with the vehicle (i.e. control). Obvious differences in size of the colon sections can be seen (see text).

PLATE 3

Studies on renal side effects of FK compounds:

Illustrated below is the set up where rats were placed in cages after administration of a water load followed by oral administration of the various FK compounds using a garage. Samples of urine were taken at particular time intervals for mineral analysis. The cummulative amount of urine was noted at each selected time interval. Urine was continously collected for 24 hours after drug administration and th pH, glucose content and protein levels were also analysed (see text).

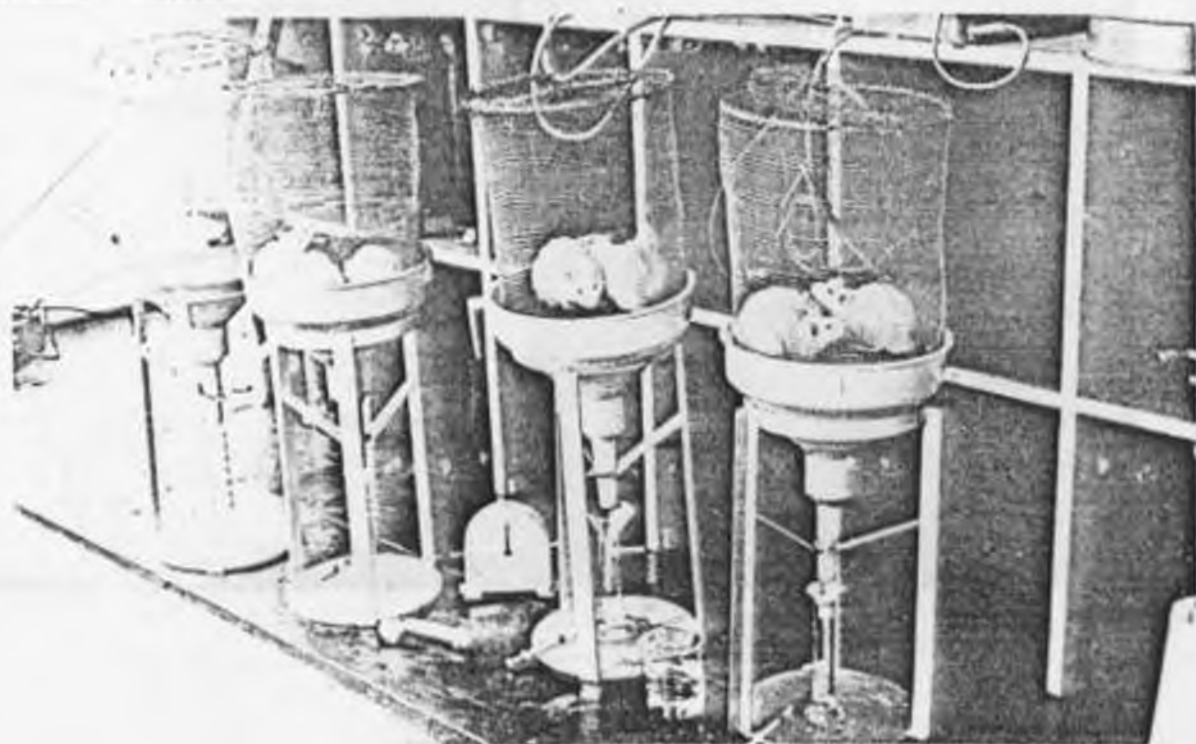
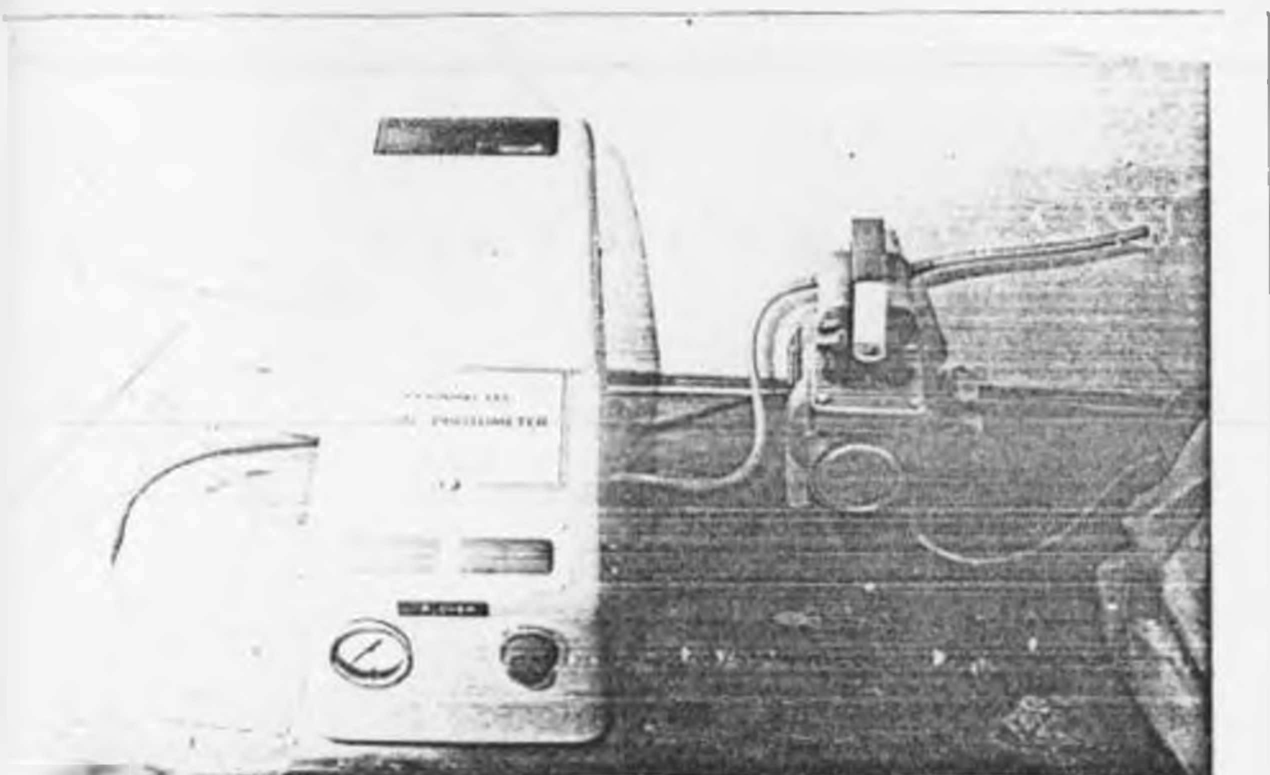


PLATE 4

Analysis of Urine Samples: Bioassay for Mineralcorticoid Activity

Below is shown the flame photometer used in the analysis of the amount of sodium and potassium in urine sampled at specified time intervals (see text).



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