ELECTROLYTIC CHANGE IN THE BLOOD AND BONES OF EXPERIMENTAL ANIMALS UNDER THE INFLUENCE OF NEWER ANTI-INFLAMMATORY AGENTS

By

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Thesis submitted for the Degree of M.D.
(Pharmacology)

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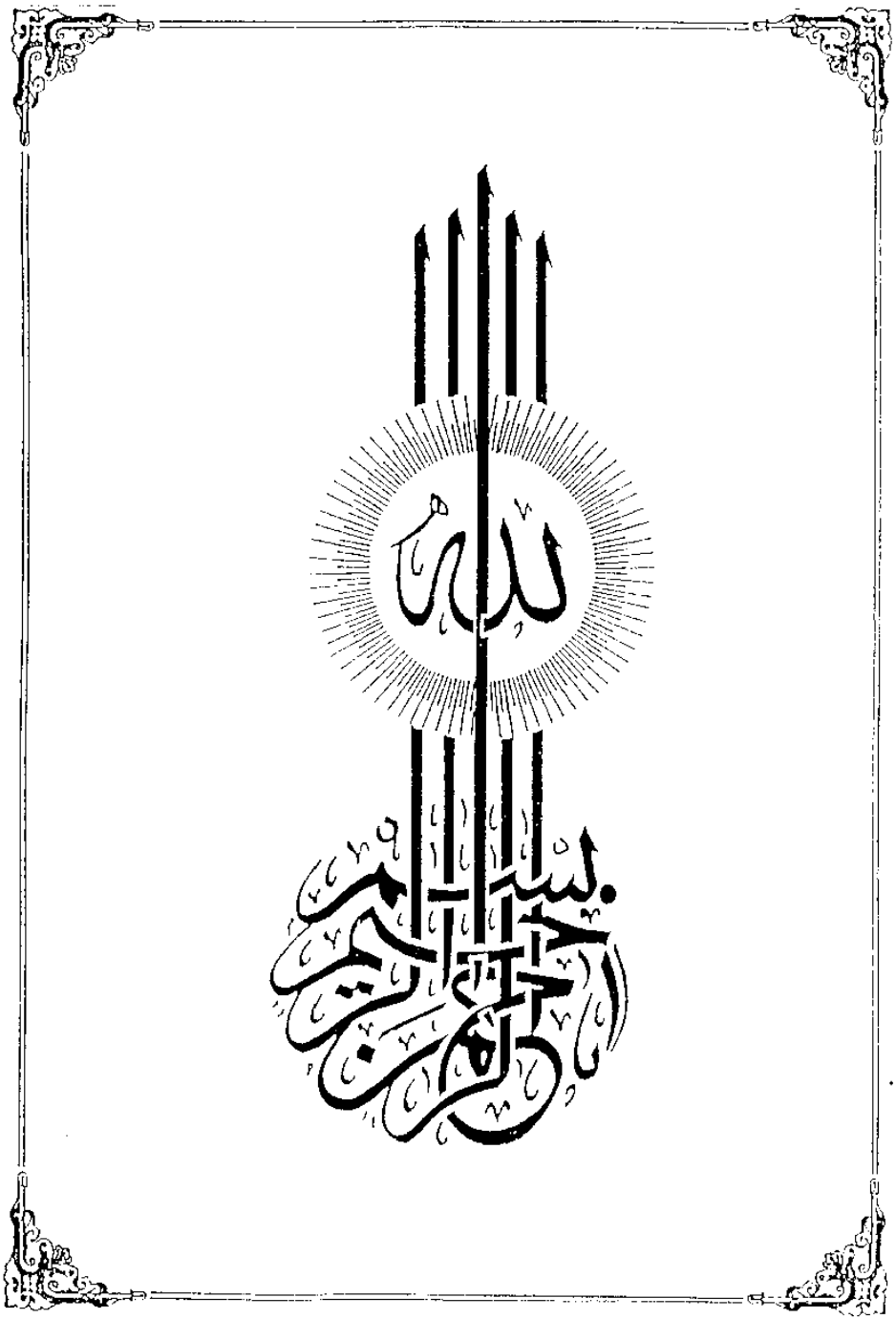
1973

Acknowledgement

I wish to express my sincere gratitude and everlasting thanks to Professor Dr. Salah Abdel Tawab, Chairman of Pharmacology Department, Faculty of Medicine, Ain Shams University, for whom I am much obliged and deeply indebted for his suggestion of the subject of this thesis, his kind approval to supervise the experimental investigations, and his proper and invaluable help throughout the work.

I wish also to avail myself of this opportunity to thank Professor Dr. Saadeya Abdel Hafize, Professor of Pharmacology, Faculty of Medicine, Ain Shams University, for her help, fruitful discussion and encouragement, along with providing me with every facility at her disposal. Her invaluable experience along with untiring and persistent efforts for which I am profoundly grateful, finally brought this thesis to a successful conclusion.

Lastly but by no means least I wish to extend my deepest obligation and cordial thanks to Dr. Mahmoud Kadri Madkour, Head of Pharmacological Research Laboratories, Faculty of Medicine, Cairo University, for his co-operative spirit, unforgettable help and experienced advice in the general arrangement and style of writing of the text of this thesis as well as in tabulation and graphic presentation of the results.



C O N T E N T S

P R E F A C E

The work on the new anti-inflammatory drugs began in the early fifties.

The cortisone drama was well known and phenylbutazone was becoming recognized as a potent anti-inflammatory agent. Management of inflammatory conditions were breaking out of the old salicylate mould.

There were overlapping periods of efforts to improve on phenylbutazone in the pyrazolone series and to better aspirin* significantly in the salicylate series; but these efforts were eventually abandoned. Good activity was developed in certain seemingly unrelated chemical series, but the activities could not be separated from unwanted acute side effects.

In the course of much semirandom screening, clear activity was found among N-phenyl anthranilic acid derivatives, flufenamic acid (Arlef) (Parke Davis) being the first compound to be introduced.

Antipyresis and inhibition of inflammatory granulomata formation in rats were demonstrated (Winder et al., 1963).

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*Acetyl salicylic acid

Mefenamic acid (Ponstan) was subsequently synthesized and became the second compound to be recommended for clinical trial (Winder et al., 1962).

Hundreds of other fenamic acids were synthesized and studied. Out of this effort came meclofenamic acid, and it was the third compound to be recommended for clinical trial (Winder et al., 1965).

In the present work, potency of different members of fenamic acid, e.g. flufenamic, mefenamic and meclofenamic together with indomethacin is determined on the electrolytes of both the blood and bone relative to that of phenylbutazone.

Compound Ciba 21,401 Ba (Glyvenol) is added to the list because it has been found to have anti-inflammatory activities and is extensively used.

Changes in electrolytes sodium and potassium of blood are extremely important since these drugs are used in the stage where, congestive heart failure, hypertension, nephrotic disease and diabetes supervene, or are common associations in our patients.

Electrolytes in bone are also determined since bone contains a large proportion of the total amount of sodium within the body (Bergstrom, 1952; Davis, Konberg and Wilson, 1952; Cheek, West and Golden, 1957) i.e. not less than half the sodium in the body.

Bone is the only tissue in which the sodium content is greater than in extracellular fluid (Harrison, Darrow and Yannet, 1936).

A good deal of attention has been focused on the role of bone in sodium and potassium metabolism (Bergstrom, 1956; Nichols and Nichols, 1956; Neuman and Neuman, 1957 & 1958).

Bergstrom and Wallace (1954) suggested that bone also participates in potassium exchange though to a lesser degree. It has also been suggested that bone sodium is labile and may act as a freely available reservoir for the preservation of the plasma sodium level (Woodbury, 1956).

In contrast, the proportion of the total skeletal calcium which is readily exchangeable is surprisingly

small (less than 1.5 g) necessitating pooling of all the bones of the experimental animals for quantitative analysis (Campell, 1968).

The goal of this effort has been to find out whether these new compounds have a pure anti-inflammatory activity or is it associated with sodium retaining properties in plasma and bone, as well as its effect on potassium in these compartments.

Previously sodium retention in plasma had become evident shortly after the introduction of cortisone (Robert and Pitts, 1952; Knowlton, Loeb, Stork, Kohler, 1953; Sprague et al., 1950).

Abd El Hafize (1960) studied the effects of corticosteroids, DOCA, cortisone, hydrocortisone (cortisol), prednisone, and prednisolone and dexamethasone on the level of sodium and potassium in the bone. In the present work we studied the effect of different groups of non-steroidal anti-inflammatory agents on sodium and potassium of both bone and plasma. A comparative study was made to elucidate which of these can induce more significant changes in the electrolytes studied taking phenylbutazone as a standard.

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

Dynamics of bone sodium:

Within recent years bone has come to be regarded as a functional depot playing a significant role in total body electrolyte metabolism rather than as a stable pool of unavailable ions (Bergstrom, 1955). Attention has been focused on the possible role of bone in relation to plasma sodium regulation (Dosekun, 1959). It has been shown that relative to other tissues, the sodium content of bone is high and undergoes constant turnover throughout life (Shohl, 1939) and that bone is the only tissue in which sodium concentration is higher than in extracellular fluid (Harrison, Darrow and Yannet, 1936). It has also been suggested that bone sodium is labile and may act as a freely available reservoir for the preservation of the plasma sodium level (Woodbury, 1956). Evidence has also been forthcoming from animal experimentation that the skeleton may gain or lose electrolytes under conditions of abnormal electrolyte metabolism (Bergstrom, 1956).

Analysis of the whole human body, although limited to a few observations has shown that the total sodium content is approximately 5000 mEq (Widdowson et al., 1951; Forbes and Lewis, 1956); of this total probably not less than half is in bone.

Information as regards human bone is sparce. In 1894, Gabriel in a very detailed study of animal bone, included two representative samples from unstated number of specimens of the humerus of man and he was the first to demonstrate considerable quantities of sodium in bone.

Klementin (1936) also conducted a comprehensive investigation on human mineral content but his material was limited to three samples of skull and two each of pelvis and humerus.

Recent reports of human bone composition have appeared (Mitchell et al., 1945) and (Forbes and Lewis, 1956). However, these latter observations have not been wholly satisfactory in that the number of samples analysed were few and the determinations were limited to two or three minerals. The largest study currently

reported is that given by Pelligrino and Farber (1958). These workers analyzed tibial bone of fifteen normal subjects for calcium, phosphorous, sodium and potassium. In a similar fashion Agna, Knowles and Alverson (1958) analyzed normal human skull, rib and ileum for concentrations of water, calcium, phosphorous, carbon dioxide, nitrogen, chlorine, potassium and sodium. The skull was found to contain significantly greater amounts of calcium, phosphorous, carbonates, and sodium and lesser amounts of water, nitrogen, chlorine and potassium than did ileum. The composition of rib was intermediate between skull and ileum. A comparative study was also carried out by Shenolikar (1966) on different species of human Indians where he found no marked difference.

However great difficulties in attempting to study alterations in bone sodium exchange in man during life were encountered, besides problems concerning the internal distribution of this ion and factors related to the body economy of sodium regulating its overall balance (Casey and Zimmerman, 1955).

It was not until the use of radio isotopes which gave more accurate information about the dynamics of

bone mineral metabolism that the potential importance of bone in sodium metabolism was appreciated (Hevesy, 1955; Neuman and Neuman, 1958).

Ever since the earlier studies with radioactive isotopes, it has been realized that bone sodium does not exchange completely within periods adequate for complete exchange in all other tissues (Kaltreider et al., 1941). Later work has repeatedly confirmed this observation in different species. For example, Bauer (1954) found in rats that only 30-40% of bone sodium exchanged with injected radio-sodium within twenty-four hours and similar values have been reported in rabbits (Davies et al., 1952) and in dogs (Edelman et al., 1952; Miller and Wilson, 1953; Miller et al., 1954). Using an external counting technique, Miller et al. (1954) were able to obtain information about the penetration of radiosodium into bone in human subjects on a diet of low sodium content and in a steady state of sodium balance. Their method was not sufficiently sensitive to attempt to follow alterations under conditions of altering sodium balance. For this reason the effects of acute sodium depletion were studied in rats by Munro

(1959) who confirmed that when sodium was removed from the body by peritoneal dialysis (according to Bergstrom and Wallace, 1954), this procedure reduced the bone sodium content with variability between young and older rats (Munro, 1959). In older rats, the decrease in bone sodium was not so pronounced although the amount of sodium available for exchange was not altered. It is evident that the transfer of sodium from bone to extracellular fluid must, in the first instance, be from the exchangeable fraction which by definition, is able to participate in ionic exchange with the extracellular fluid. These results suggest therefore that, after depletion, some sodium in bone which was not originally available for exchange had become exchangeable so that the absolute amount available remained constant.

The interest in the participation of bone in sodium and potassium metabolism has mainly been focused on the potential of this structure as a reservoir for the electrolytes, from which they could be supplied in case of need and where they could be stored in periods of surplus. In most of the published studies the investigators have

concentrated on the significance of bone as a source of extrasodium mainly during metabolic acidosis (Bergstrom, 1952; Nichols and Nichols, 1953 & 1958; Levitt et al., 1954 & 1956). Metabolic acidosis and sodium depletion will condition a movement of sodium from bone, being rich in its sodium content.

Bergstrom (1955) was the first to show that significant loss of sodium from bone resulted from the induction of systemic acidosis in animals. In these experiments, as well as in sodium deprivation studies, he demonstrated that the loss of sodium from bone was as much as 30 to 50% of that originally present. This indicated that a large part of the skeletal sodium exists in a form which can be liberated, at least under certain conditions.

Considerable loss of sodium and potassium from bone in electrolyte depletion without acidosis has also been found (Bergstrom, 1954 & 1956; Edelman, Baden and Moore, 1954; Nichols and Nichols, 1955 & 1956; Woodbury, 1956; Munro Satoskar and Wilson, 1957). Bergstrom (1955), Nichols and Nichols (1956) and Levitt et al.

(1956) demonstrated lowering of bone sodium content after loss of sodium from body and vice versa, sodium content may rise after sodium loading. Nichols and Nichols (1957) were able to demonstrate a gain in total bone sodium by chronic sodium loading and after parathyroidectomy (Nichols and Nichols, 1958).

Also the effects of alterations of the concentration of sodium in the serum upon the content of sodium in bone have been studied in rats in experiments of relatively short duration. Hyponatraemia of short duration produced either by water loading in the presence of vasopressin or by peritoneal dialysis against glucose solutions was not associated with a significant fall in bone sodium. Similarly hypernatraemia produced by urea loading and restriction of water intake was not associated with a significant rise in bone sodium. This was demonstrated by Winters et al. (1958) who suggested thus that alterations in the concentration of sodium in the serum or in the volume of the extracellular fluid do not cause movements of bone sodium in experiments of short duration.