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PHYSIOLOGIC AND BIOCHEMICAL CHANGES IN DEHYDRATION AND ITS EFFECT ON PHARMACOKINETICS OF ACETAMINOPHEN

by

TASNEEM AHMAD
B. Pharm., M. Pharm.

THESIS

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(Tasneem Ahmad)

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1. LITERATURE REVIEW

1.1. INTRODUCTION

All living bodies fundamentally need water for their survival. The human body contains almost 60% of water by weight.[1] The water requirements are fulfilled in three ways; firstly, as drinking water; secondly, an appreciable quantity of water is consumed through solid food and thirdly, through the water generated by biochemical processes. Not only every cell of the human body contains a good quantity of water but, except the external skin, all the tissues lie constantly in an aqueous environment. It is because of the water present in the blood that each cell receives its proper food.

Water is essentially required for the growth of the body as well as for the regulation of metabolism which is responsible for providing the vital energy for life. At the same time water helps to eliminate the products of metabolism occurring in the tissues.

The excretion of water from the body occurs through the urine formation in kidneys, sweating and expiration. Thus the body needs to recover this constant loss of water by suitable means. Since we do not drink water in the same frequency as we inhale the air, the body undergoes dehydration gradually till a stage is reached when osmolality exceeds the limits and we feel thirst through osmoreceptors This motivates us to drink

water so as to replenish this deficiency. The term voluntary dehydration[2] refers to that short period of time during which hyperosmolality develops prior to the drinking of water. The constant deficiency of water in the body results in clinical dehydration which is an important state of human physiology.

As the universe is highly rich in water, its scarcity hardly becomes the main cause of dehydration. However, humans may be subjected to dehydration in particular circumstances such as being stranded in the sea, trapped in a desert or when the transportation/availability of water is severely disturbed during a war or by a natural calamity In such conditions the dehydration may be fatal to the body system. Another cause of severe dehydration is the extraordinary discharge of water from the body as in the case of excess bleeding or due to disease states such as polyurea diarrhoea, vomiting and cholera. The dehydration in the diseased states may be very harmful because it is generally associated with the loss of electrolytes. The deficiency of water in the body may arise in the following cases as well:

1. Mouth or throat or oesophageal obstruction.
2. Restricted intake after gastro-intestinal operations.
3. Cerebrovascular disease state.

In addition to the above mentioned cases dehydration is sometimes deliberately induced during fasting[3,4] whereby individuals refrain from drinking water from 12 to 24 hours.

Dehydration often causes stress and weakness in addition to various physiologic and biochemical changes in the body. There is a significant deficiency of water in the fluid compartments as well as in the total body fluid which is the most serious consequence of dehydration leading to disturbance in the distribution and equilibrium of biochemicals and electrolytes in the body. Some other effects include the malfunctioning of metabolic enzymes and kidney.

In view of the varied effects noted above and the resulting abnormalities it is obvious that dehydration may likely disturb the absorption, distribution, metabolism and renal excretion of drugs administered in various ailments. Thus it is necessary to have a careful consideration in the choice of the drug and in the determination of rational for the required dosage regimen.

The present study has been undertaken to evaluate the effects of dehydration on physiologic and biochemical parameters in human volunteers who refrained from drinking water for upto 24 hours. Acetaminophen has been selected as a model drug to evaluate its pharmacokinetic behaviour in the state of water deprivation. It needs to be emphasized that no comprehensive work on dehydration in humans has so far been reported in the literature.

1.2. PHYSIOLOGIC AND BIOCHEMICAL CHANGES IN WATER DEPRIVATION

The important physiologic and biochemical changes resulting from water deprivation in humans and animals may be summarised as follows:

1.2.1. Weight Loss

Weight loss during water deprivation has been accepted as a physiologic reality in humans and animals. It has been reported for rats,[5-7] mini pigs,[8] dogs,[9] horses,[10,11] sheep,[12] fowl,[13,14] guinea pigs, hamsters and gerbils.[6] Weight loss due to water deprivation in rats and guinea pigs is equal to the total body water loss.[6] Its manifestation in rats may be noticed as early as 8 hours after water deprivation.[15] However, within 24 hours it crosses the 5% loss limit, which is clinically a crude index of fluid deficit.[16] In dogs, this much loss does not appear to produce other clinical signs of fluid loss.[9] Some reports recently mentioned a significant loss in body weight in humans after short term water deprivation.[17,18]

1.2.2. Fluid Deficit

The most profound primary effect of water deprivation, which initiates an unending cycle of physiologic and biochemical changes in the body, is the total body fluid deficit. First it manifests itself in extracellular fluid compartment, which in turn affects intravascular fluid and then the extravascular one. Secondly it appears in the intracellular fluid compartment.

Changes in different fluid compartments due to water deprivation have assumed an issue of high interest for researchers. Only 24 hours' water deprivation produces significant decrease in the plasma volume of rats,[5] whereas no change occurs in the total erythrocyte volume. The major portion of 3% loss in total body water occurs in the intravascular fluid. Significant losses in plasma volumes of humans,[19] and in the intracellular fluid, extracellular fluid and plasma volumes of horses,[11] due to water deprivation have been recorded. However, in horses, in relation to body weight there was no reduction in blood volume. On the other hand, the losses in extracellular fluid and plasma volumes were proportionally more significant than the body weight loss. The total fluid deficit was found proportionally equal in the intracellular and extracellular compartments. Early water deprivation causes reduction in plasma volume of rats[6,20] and the body defends extravascular fluid to keep it unchanged at the expense of plasma volume. However, at one third loss of the plasma volume, body defends it at the expense of extravascular as well as intracellular compartments.

1.2.3. Hyperosmolality

Hyperosmolality of serum and urine in water deprivation is well documented.[9,11,15,21-23] Hyperosmolality of plasma is a logical outcome of increased concentration of electrolytes and proteins due to reduction in plasma volume during water

deprivation. The first measurable change recorded only 4 hours after water deprivation, is hyperosmolality of plasma.[15] Osmolality increases with the time course of deprivation. However, like plasma volume it becomes stabilised after 48 hours and remains unchanged upto 72 hours. Hence a significant inverse correlation exists between plasma volume and plasma osmolality, and through a suitable regression equation plasma volume may be predicted by checking only the plasma osmolality.[21] Progressively increasing values for plasma osmolality cause fluid shift from the extravascular compartment into the plasma, which ultimately reduces the fluid in the intracellular compartment.[20] To keep the plasma osmolality stabilised at some maximum normal value, the electrolytes are excreted through kidney at higher rates than the normal. The excretion of electrolytes depends upon the concentrating ability of urine which depends upon the ratio of urine and plasma osmolalities, provided the ratio does not exceed the limits which are specific for different animals and species. As a consequence of hyperosmolality the excretion of antidiuretic hormone is increased and thus the urine flow-rate is decreased. Higher excretion of electrolytes in low volume of urine makes the urine concentrated and hyperosmolal. The maximal values for urine osmolality and specific gravity and the ratio of osmolalities of urine and plasma during dehydration have been recorded.[9,24-26] The maximal urine osmolality in humans during water deprivation depends upon many non-renal factors such as age, state of hydration, small changes in urine

solute excretion, changes in renal blood flow, air temperature and humidity.[27,28] However, no effect of age has been observed on the urine concentrating ability of rats.[29]

1.2.4. Hematological Changes

An increase in the hematocrit value resulting from the reduction in intravascular fluid is well known.[6,10,30-32] Not only the water deprivation may cause an increase in the hematocrit value, but the food and water or only the food deprivation may also cause it.[21] Thus the value can not be used as a consistent index of hemoconcentration/plasma volume during water deprivation,[9,21] although some workers have proposed to use it for this purpose.[6,33] It has been pointed out that due to high urine osmolality, as happens during water deprivation, life span of red blood cells might be reduced or these cells may be destroyed to some extent.[34] Accordingly, the osmolality of plasma, interstitial fluid, and tubular urine increases progressively from renal cortex to medulla. Thus when the kidney is excreting a concentrated urine, the red blood cells passing through the renal medulla are subjected to greatly increased osmotic pressures, a condition that is believed to lead to their early senescence. On the contrary, by means of reduced water intake high osmolality in urine and plasma was produced and survival and prolonged life span of red blood cells were observed.[35] It was also noted that due to water deprivation reticulocyte count is decreased and erythropoiesis is supressed. In another study on rats

the reduction in red blood cell count was found proportional to the weight loss in 3 days after water deprivation which may lead to the depression of the bone marrow function.[20]

1.2.5. Changes in Serum Proteins

During water deprivation scarcely any dry food can be taken and it, therefore, accompanies with food deprivation to some extent.[36] Even the glucopronic acid stimulus fails to evoke any increase in food intake in water deprived rats.[37] Thus during water deprivation there must be a loss in total protein content due to its lower intake as well as continuous metabolism for the body needs. It is observed that water deprivation causes protein deficiencies in cell nucleus and cytoplasm of neurons and gila.[38] However, in order to preserve the volume of the extravascular fluid compartment in initial stages of water deprivation there is proportionally higher loss of plasma volume than the loss of blood protein content. Thus protein concentration in plasma seems to be increased.[5] There exists a significant inverse correlation between the plasma volume and plasma protein concentration,[21] whereas this correlation observed in dogs and horses is not absolutely quantitative.[9,11]

Like the urine osmolality and plasma volume, no change is observed in the total plasma proteins during 48-72 hours of water deprivation.[15] This further confirms the preservence of plasma volume during the period of dehydration at the expense of

extravascular/intracellular fluid. After thermal water deprivation an increase in albumin content of laboratory rats has been recorded, whereas no such excess in albumin has been observed in desert rats.[39]

1.2.6. Changes in Electrolytes

Water deficiency produces significant changes in the kinetics of electrolytes in the body. All these changes account for (a) to excrete out the excess electrolytes particularly sodium and to maintain the toxicity in accordance with the lowered water content, (b) to minimize the water excretion from the body by retention of extra sodium ions and other electrolytes to a tolerable limit, and (c) to preserve the extracellular, particularly blood volume, by excretion of cellular potassium ions and consequently fluid shift from intracellular to extracellular compartment. The mechanism of excretion, retention and redistribution of electrolytes in animals and species during dehydration may vary significantly. Many workers in this field have observed a peculiar behaviour for sodium during water deprivation, which is the so called dehydration reaction.[20,31,40-44] In this condition the sodium concentrations are retained in plasma at higher levels than those in the normal condition, i.e. with little excretion of the ions and increased elimination of potassium ions. This condition is developed in the later stages of water deprivation. It has been demonstrated that in water deprivation there is a priority to excrete cellular potassium over extra-cellular

sodium, thus allowing more water to be withdrawn from the intracellular compartment of the body and hence preserving the extracellular volume to some extent. High renal excretion of sodium ions is found in early stage of dehydration, whereas high excretion of chloride ions has been observed throughout the water deprivation period.[45,46] Thus a net loss of electrolytes and water occurs due to water deprivation. Similar situation has been observed for rabbits and sheep.[47] If the deprivation remains unaltered for a longer period, the sodium and potassium levels of the whole body are increased.[48,49] Horses exhibit high sodium and chloride plasma levels after water deprivation while no change occurs in the potassium, phosphorus, calcium and magnesium levels.[10] In another study on the horses high potassium and unchanged sodium and chloride plasma concentrations were reported.[11] The rats dehydrated by keeping them on sea water for long periods (45-70 days) excreted very large amounts of chloride ions in urine. During this period comparatively small shrinkage of the kidneys occurred than the body weight loss and adrenal glands were found at their original size.[50] Higher potassium circulation in humans occurs without any muscular contraction after thermal dehydration.[51]

1.2.7. Changes in Brain, Kidneys and Lungs

The significant loss in intravascular volumes, which results in hemoconcentration and hyperviscosity, needs extra pumping force by increased heart rate for blood supply even though

there is a decrease in the volume level.[8] The vital parts of a body like heart, brain and adrenals receive maximum supply of blood whereas the selective vasoconstriction reduces the blood supply towards kidneys, lungs, liver, skeletal muscles and gastrointestinal tract.[8,52] The renal vascular resistence is increased because of the increased viscosity of blood due to hemoconcentration and reduced blood supply to kidney in dehydration.[53] Water deprivation for 48 hours seems to produce no change in the potassium level of brain in rats, since the blood brain barrier helps to ensure the water electrolyte balance of the brain.[21] The reduction in pulmonary blood volume is proportionally far greater than the reduction in total blood volume.[5] Low urine formation or reduced urine flow rate in dehydration is one of the much pronounced dehydration effects in animals and humans.[17,18,54,55]

1.2.8. Acid-Base Status of Blood

Some early studies showed significant decrease in plasma bicarbonate concentration in water deprived humans.[56,57] Similarly contraction alkalosis was reported following the water deprivation in previously edematous patients after 2-3 days' treatment with ethacrynic .c acid.[58] However, some later studies on rats could not confirm such changes.[32,59] On the contrary the rats showed a tendency (statistically not significant) for decrease in blood pH and PCO_2 as well as plasma bicarbonate concentration.[59] On the average the rats did not show any change in acid-base status of blood after 4 days' water deprivation. In another study

after 12 days' water deprivation no significant change was found in acid-base status of the blood of rats, particularly the pH, the base excess and blood buffering capacity remained totally unchanged throughout the period.[33] However, an increase in PCO_2 and bicarbonate was noted on the 10th day of water deprivation, which disappeared at the end of the 12th day.

1.2.9. Enzymatic and Hormonal Changes

Water deprivation is a sort of stress which always results in different types of enzymatic and hormonal activities to combat with bodily changes. During dehydration neoendorphin, dynorphin, leucine-enkephaline are depleted from neurohypophysis of rats.[60,61] Water deprivation is also found to be a stimulus for noradrenaline synthesis and dopamine utilization in albino rats[62] as well as to the hypothalamoneurohypophysial system resulting in the release of stored hormones (vasopressin and oxytocin) from the neural lobe.[63-66] For the extrusion of these hormones a hypothesis was proposed in terms of exocytosis or in terms of intracellular disruption of hormone stores.[67] It was concluded that the limiting membranes of these hormone stores or their residues after depletion are metabolized by five lysosomal enzymes.[68] During dehydration in neural lobe some significant increase in the lysosomal enzymes acid; phosphatase, β-glucosidase, β-glucuronidase, β-galactosidase and N-arylamidase has been found. Water deprivation is responsible for the fall

in activity of ornithine decarboxylase in mice,[69] decrease in the yield of microsomal proteins,[70] body amylase and creatinine clearance without affecting the renal back diffusion.[67] It is also a potent stimulus for the release of antidiuretic hormone (ADH)[72] and increase in plasma arginine-vasopressin concentration in rats[52,73-79] and dogs.[80]

1.3. EFFECT OF WATER DEPRIVATION ON PHARMACOKINETICS

The effect of starvation on the microsomal enzymes of liver, which are responsible for metabolism of foreign compounds, has been extensively studied in rats[81-83] and humans[84]. The starvation is believed to cause an acute loss of microsomal enzyme proteins. The oxidation pathway is affected more than the reductive pathway and starvation may result in an activation of the reduction of nitro and azo groups.[81] Even short periods of starvation markedly reduce the metabolism of some foreign compounds.[82-84] The increasing susceptibility of acute poisoning with lead and antimony in short-term water deprivation has been reported in humans.[85,86] This has generated much interest in evaluating the effect of water deprivation on hepatic microsomal enzymes responsible for drug metabolism.[87-89]

In-vitro studies involving hexobarbital and aniline metabolism by hepatic microsomal enzymes of rats deprived of water for 24 hours (food ad lib) show that hexobarbital metabolism is significantly lowered as there was an increase in the mean

sleep time. On the other hand, the metabolism of aniline by hepatic microsomal enzymes was significantly higher in water deprived rats. This finding is explained on the basis of formation of different binding spectra by hexobarbital and aniline with cytochrome P-450.[87] Contrary to the findings on rats, studies using mongolian gerbils show enhanced metabolism of hexobarbital in water deprived animals.[88] Thus, both the nature of the drug and the species involved play an important role in determining the outcome of water deprivation on hepatic microsomal system. An interesting observation has been made regarding the disposition of lithium in water deprived rats.[89] The urinary clearance of lithium is significantly lowered upon water deprivation only if the effect of lithium (polyurea) is pronounced. These studies aroused much interest in the effect of water deprivation on pharmacokinetics of drugs. It was observed that the altered pharmacokinetics of a drug might necessitate some changes in dosage regimen for a patient in the water deprived state. Significant changes in pharmacokinetics of drugs during water deprivation have recently been reported.[90-93] The first study of this kind in humans describes the effect of water deprivation (highly restricted water intake) on pharmacokinetics of chloramphenicol after oral administration in healthy human volunteers.[90] The total bioavailability of chloramphenicol in this condition was unaltered but the absorption half-life was decreased by 66%. Significant changes in peak renal excretion rate and time at which peak excretion occurs were also observed.

In a similar study in humans, significant increase in biological half-life of amoxycillin has been reported, which may alter the therapeutic efficacy of the antibiotic and necessitate a new rational dosage regimen for patients with variable degree of dehydration.[18]

Besides the work on humans, some recent studies show significant changes in disposition kinetics of drugs in rats.[91-93] Following four days of water deprivation the rats showed a decrease in total body clearance of gentamycin with an accompanying increase in plasma and tissue levels of the drug. This was caused by a significant decrease in the distribution volumes of central and peripheral compartments.[91] Similarly in another investigation on rats, 36 hours of water deprivation led to a significant decrease in the total body clearance and thus increased half-life of salicylic acid.[92] In a very recent study on rats, 96 hours of water deprivation caused a pronounced shift in the metabolism pathways of acetaminophen towards faster glucuronidation, thus resulting in a significant decrease in the percent excretion of the unmetabolised drug.[93]

1.4. ACETAMINOPHEN

Acetaminophen, the N-acetyl conjugate of para-aminophenol is one of the most widely used non-narcotic analgesic and antipyretic.[94] It is used to relieve moderate pain like headache, mylagias, arthralgias and other pains arising from muscles and

joints and peripheral nerve affections. It is less toxic than the salicylates and does not produce the methemoglobinemia, agranulocytosis and anaemia.[95] Acetaminophen is quite a safe drug of choice for mild pains, provided it is administered to the persons with normal hepatic functions in therapeutic doses. Because of this it has assumed the status of a common household analgesic. However, an overdose, at least 15 times more than a single adult dose of 650 mg, leads to hepatic necrosis. Ingestions of large doses. 10-15 g. results in saturation of glucuronide and sulfate pathways and thus enhances P-450 mixed oxidase function system, producing reaction intermediates. These intermediates, after depletion of glutathione reserves, combine with cell macromolecule to produce hepatoxicity.[96]

1.4.1. Absorption

Acetaminophen dissolves rapidly in gastric fluid or acidic medium. A tablet formulation with 0.33 minutes of disintegration time in gastric fluid requires only 1±1.73 minutes for 50% and 3.0±0.0 minutes for 75% dissolution in 0.1N HCl in a basket stirrer assembly.[97] Acetaminophen on dissolution exhibits slightly acidic pH and its pka is 9.5.[98] After oral administration it is absorbed rapidly and completely. As revealed from its pKa value, its absorption primarily occurs in small intestine, however, it is also absorbed from stomach to a considerable extent.[99] Its absorption is dissolution rate limited.[97,99] Absorption rate and thus the time of peak are found to be a function of dosage

formulation and gastric emptying rate[100] which is closely related to the plasma level profile.[101] The plasma peak levels after oral administration of tablet dosage form occur in 40-60 minutes after the administration, whereas only 30 minutes are required for syrup doses.[99] The physiological availability of different commercial dosage forms is insignificantly different.[102-104] On evaluation of comparative bioavailability the three commercial acetaminophen tablets were found to have almost the same physiological availability. Whereas differences in the blood and plasma levels of acetaminophen after administration of various formulations have been observed.[105,106]

1.4.2. Biological Half-Life

Disposition of acetaminophen has been thoroughly investigated and is nonlinear from both, dose dependence and time dependence point of views.[107] The elimination of acetaminophen follows first-order kinetics.[108] The clearance of acetaminophen from the blood at any concentration decreases following a toxic dose. This occurs because high doses of acetaminophen causes hepatoxicity, which reduces the ability of liver to metabolize the drug.[109] The plasma half-life of the drug in humans is between 1 to 3.5 hours.[97,110-115]

1.4.3. Protein-Binding

To interpret disposition kinetics, particularly the renal clearance, a knowledge of plasma or serum protein-binding

characteristics is of utmost importance. The protein binding of acetaminophen and its two major metabolites, i.e. acetaminophen glucuronide and acetaminophen sulfate, is not much appreciable within the range of 5% to 21%.[115-117] It has been noted that upto 43% could be bound to plasma protein after toxic doses of the drug.[116] In a very recent study[118] the serum protein-binding of acetaminophen and its glucuronide conjugate has been found to be very limited as reported earlier.[115-117] These investigators[118] found that protein-binding of acetaminophen and glucuronide conjugate are = 20% and 10% respectively. Unlike the previous findings[115-117] acetaminophen sulfate binding to serum protein is higher > 50%. The binding of acetaminophen and its two major conjugates in serum from rats pretreated with acetaminophen was 23%, 5% and 65% respectively.[118]

1.4.4. Renal Excretion of Acetaminophen

In normal therapeutic doses acetaminophen does not affect creatinine clearance in humans.[119] Glomerular filteration rate and other renal function indices also remain unaltered in experimental animals.[120-122] Renal clearance of unchanged acetaminophen is low[96,118,123] and is positively correlated with urine flow-rate[96,118] It is independent of acetaminophen concentration in the serum,[118] urine pH and its flow rate in dogs.[117] As revealed by limited renal clearance of unmetabolised acetaminophen, kidney does not play an appreciable role in its elimination.[115,124] After studies with an isolated rat kidney 92% of the excretion was accounted for the unchanged drug.[125]

Renal excretion of acetaminophen apparently follows a first-order kinetics.[126,127] In humans the renal clearance ratio of acetaminophen with creatinine clearance is quite small (0.058) which reflects extensive renal tubular reabsorption since the serum protein-binding of the drug is very limited.[118]

1.4.5. Metabolism

As already described (section 1.4.4) the renal disposition of acetaminophen is very small. It is eliminated from the body primarily by metabolism in the liver. In patients with hepatic disorders like necrosis or cirrhosis biotransformation is markedly affected resulting in significantly decreased elimination rates and thus biological half-lives.[96,128] Acetaminophen is biotransformed mainly by glucuronidation and sulfation pathways.[96,126,129,130] It has been well documented that glucuronidation and sulfation in humans are capacity limited.[126,129,131] Both the conjugation pathways follow Michaelis-Menten kinetics, whereas oxidation of acetaminophen is by apparent first-order kinetics.[127,128] On saturation of glucuronidation and sulfation, reactive moities are produced due to oxidation through P-450 system. These moities react with hepatic glutathione, and on depletion of it combine with cell macromolecules to produce hepatoxicity.[96] The renal contribution to acetaminophen conjugation quantitatively is negligible in humans, since the elimination of acetaminophen by biotransformation is not impaired in anephratic patients.[115] Studies in isolated rat kidney and

isolated rat kidney cells have shown that glucuronidation and sulfation take place in the tissues of kidney at a very low rate.[120-122,125,132] In humans, the glucuronide conjugate constitutes 45%-60% while the sulfate conjugate is about 25%-30%.[108,111,112,129,133,134] The amount of drug eventually excreted as each conjugate gives an index of the quantitative importance of each pathway in the metabolism of acetaminophen.

1.4.6. Renal Clearance of Acetaminophen Conjugates

The free OH group of acetaminophen causes severe tubular reabsorption and makes the urine clearance low as well as urine flow dependent. Liver metabolizes the drug to eliminate it from the body rapidly. The free OH group is blocked by presence of a conjugate group and thus tubular reabsorption is blocked and renal clearance of the drug is restored through conjugates. The metabolites are excreted as such in urine.

The renal clearance of acetaminophen glucuronide and acetaminophen sulfate has been studied in humans and animals.[96,108,118,123,135] These studies employed acetaminophen rather than the conjugates themselves. Since it is reported that renal tissues may also form these conjugates at a very low rate, all the determined values represent apparent renal clearances instead of the actual renal clearances. It has recently been reported that the renal clearance of acetaminophen sulfate decreases with an increase in its serum concentration and has a

protein-binding more than 50%.[118] These facts demonstrate that acetaminophen is subject to renal tubular secretion. Strong evidence for tubular secretion for acetaminophen glucuronide is not reported. In an another study acetaminophen glucuronide is reported to be subjected to both tubular secretion and reabsorption.[117] An appreciably high positive correlation between renal clearance of acetaminophen glucuronide and the sulfate in normal humans suggests that the secretion of both conjugates is renal blood flow-rate dependent.[118]

STUDY OBJECTIVES

In the light of the literature review presented in the preceeding sections it is evident that:

I: Dehydration brings about many physiologic and biochemical changes in the system, which have been studied upto a large extent in the animals particularly the rats. However, except some scattered reports covering very few physiologic and biochemical aspects no comprehensive work is reported in the literature. Thus there is a need to carry out some work in this direction.

II: Some recent reports mentioning significant changes in the disposition of drugs necessitate the study of effects of dehydration on pharmacokinetics of clinically important and commonly used drugs in humans.

III: The selection of acetaminophen seems imperative as it is one of the widely used clinically important drug and a recent report in rats has indicated some change in the metabolic pathway of acetaminophen during the state of dehydration.

In view of the above mentioned points the present study has been under taken with the following objectives:

1. To study the physiologic and biochemical parameters in humans after water deprivation for specific periods. The proposed parameters include weight, hematology, serum proteins, enzymes, renal and hepatic function, acid-base status, gas content of blood and electrolytes levels in blood with their renal clearances.

2. To evaluate the pharmacokinetics of acetaminophen in humans (after oral administration) in normal condition and in the state of dehydration.

3. To determine the effect of dehydration on the renal excretion of the major metabolites of acetaminophen i.e., acetaminophen glucuronide and acetaminophen sulfate.

2. EXPERIMENTAL

2.1. MATERIALS AND APPARATUS

2.1.1. Chemicals and Reagents

Standards: acetaminophen,[1] metabolites;[2] acetaminophen glucuronide and acetaminophen sulfate, theophylline.[3]

Solvent system: acetonitrile,[4] methanol,[5] sodium sulfate,[6] and phosphoric acid.[7]

Reagents: perchloric acid,[8] acetic acid,[9] polyethylene glycol,[10] ponceau-S dye,[11] tris-barbital buffer[12] pH 8.8, and heparin.[13]

Biochemical reagent kits:[14] 3306 blood glucose, 3311 chloride, 14328 CK NAC activation, 3385 creatinine, 14329 GOT (ASAT), 1430 GPT (ALAT), 3317 hemoglobin, 3321 hemoglobin, 3321 total lipids, 3327 total protein, 14341 triglycerides, and 3341 urea.

Control sera:[14] 15015 Pathonorm L, 15208 Seronorm protein and 15234 Autonorm.

Drug: Calpol[15] (Commercial acetaminophen suspension, 120mg/5ml)

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1. Reference standard, Smith Kline and French Laboratories, Karachi,Pakistan.
2. McNeil consumer product Company, Washington, U.S.A. (through Dr.Sarfaraz Niazi and Dr. Donald T. Jung, University of Illinois, Chicago).
3. Reference standard, Nicholas Company, Karachi, Pakistan.
4. Chromasolv, 34851, Riedel-De Hean Ag Seelze-Hannover.
5. Pure exicated, 34860, Riedel-De Hean Ag Seelze-Hannover.
6. 13464, Riedel-De Hean Ag Seelze-Hannover
7. GR, 3028583, E.Merck, West Germany
8. GR, 3047933, E.Merck, West Germany

Contd..

9. GR, 3047139, E.Merck, West Germany.

10. 5005, Helena Laboratories, Beaumont, TX., USA.

11. 5526, Helena Laboratories, Beaumont, TX., USA.

12. 5805, Helena Laboratories, Beaumont, TX., USA.

13. A15F, Leo Pharmaceutical Products, Ballerup-Denmark.

14. Diagnostica Merck, E.Merck, West Germany.

15. Batch No.1940, Burroughs Wellcome Company, Karachi/Pakistan.

2.1.2. Materials

Venoject blood collecting system,[1] syringes,[2] three way stop cock,[3] polypropylene microcentrifuge tubes[4] (100µl and 1000µl), reverse phase HPLC column,[5] electrophoresis cellulose acetate plates,[6] and membrane filters.[7]

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1. Plain, silicone coated, Terumo Corporation, Tokyo, Japan.
2. Terumo syringes, Terumo Corporation, Tokyo, Japan.
3. Top surgical Manufacturing Company, Tokyo, Japan.
4. Fisher Scientific Company, Pillsburgh, Pa., USA.
5. µ Bondapack C_{18}, Waters Associates, Milford, Mass, USA.
6. 3014, Titan III, Helena Laboratories, Beaumont, TX., USA.
7. Type WCN, Whatman Limited, Maidstone, England.

2.1.3. Instrumentation

Liquid Chromatograph,[1] Spectrophotometric variable detector,[2] data processor,[3] electrophoresis apparatus,[4] densitometric scanner,[5] autoanalyzer for enzymes,[6] blood gas and acid-base analyzer,[7] flame photometer,[8] centrifuge,[9] UV spectrophotometer,[10] pH meter,[11] vortex mixer,[12] hematocrit centrifuge,[13] and ultrasonic bath.[14]

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1. LC-5A, Shimadzu Corporation, Tokyo, Japan.
2. SPD-2A, Shimadzu Corporation, Tokyo, Japan.
3. C-R 1B Chromatopac, Shimadzu Corporation, Tokyo, Japan.
4. TITAN, Helena Laboratories, Beaumont, TX., USA.
5. 1202, Helena Laboratories, Beaumont, TX., USA.
6. PCP 6121, Ames-Pacer Cadit, Eppendorf, West Germany.
7. BME - 33, Radiometer, Copenhagen NV., Denmark.
8. FP-40P, Fisher Scientific Co., Fair Lawn, NJ., USA.
9. SS-1, Ivan Sorvall, Inc., Norwalk, Conn., USA.
10. UVD Spectronic-21, Baush and Lomb, NY., USA.
11. PW-9418, Philips, England.
12. K-550-GE, Scientific Industries, Inc., NY., USA.
13. Compur M 2100, Compur Electronic GmbH, Munchen 70, West Germany.
14. F.S.200, Decon Laboratories Ltd., Sussex, UK.

2.2. HUMAN EXPERIMENTAL PROTOCOL

2.2.1. Panel Composition

The panel of human subjects consisted of ten healthy adult male volunteers, weighing between 59 kg to 69 kg and a height range of 163 cm to 178 cm. The age of the volunteers varied from 21 to 26 years. Details of volunteers are given in Table I. All the volunteers participated in the water deprivation study to evaluate the accompanying physiologic and biochemical changes. The four volunteers (No.7 to 10) did not take part in the acetaminophen study.

2.2.2. Selection of Volunteers

The panel members were given a general medical examination to establish good health. The following selection criteria were used for this purpose:

1. No history of allergic tendencies and reaction to the analgesics and acetaminophen.

2. Normal blood counts, normal liver and kidney function tests, and without any abnormality in physiology and in urine and blood analysis.

3. No treatment taken nor any drug used for atleast a month prior to the study. An earlier exposure to acetaminophen was, however a must.

4. Absence of any chronic disease or any pathological state.

TABLE I

DESCRIPTION OF VOLUNTEERS PARTICIPATED IN THE STUDY

NO.	Subject code	Age (years)	Weight (Kg)	Height (M)
1	MA	25	64.75	1.70
2	MT	24	69.00	1.70
3	AB	24	62.5	1.68
4	AF	24	63.0	1.65
5	AS	22	66.5	1.80
6	KM	24	66.75	1.62
7	YK	25	67.5	1.73
8	MN	26	68.5	1.73
9	KT	23	59.0	1.75
10	MI	23	60.5	1.65

2.2.3. Restrictions Imposed

The selected volunteers, a week prior to the study, were advised to maintain their daily fluid intake around two litres and not less than one and half litre per day. They were told with emphasis to take water as early as the body develops a response for drinking and not to remain in the thirsty state in any case. They were also instructed to report to the investigator any intercurrent illness and the treatment taken, which might enable the investigator to make necessary adjustments in the procedure or the whole volunteer study program. Fortunately, no such situation arose uptil the end of the study.

2.2.4. Procedure

i) Control studies in the normal living state: The volunteers were asked to report to the investigator in the morning at 7.00 a.m. after an over-night fast. After urinating at 6 O'clock in the morning to clear their bladders the volunteers were instructed to take 250 millilitres of water. Immediately after reporting at 7 O'clock, a blood sample of 20ml was taken for hematologic and biochemical examination. One hour after the blood sampling, the volunteers collected and submitted their urine voids for biochemical analysis and renal clearance tests. The time of the urine sampling was so adjusted that the previous blood sample time became exactly at the mid-point of two urinations. After measuring the void of each volunteer, 10ml sample was preserved for testing and the rest was discarded. These

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blood and urine samples were also used as blank in the acetaminophen study. Afterwards a single dose of 25ml Calpol suspension (600mg acetaminophen) was administered at 8.30 a.m. to the subjects. The panel members were instructed to drink 150ml of water alongwith their dose. All the subjects were asked to refrain from taking any food or drinks until about two hours after the ingestion of the dose. Blood samples, each of 1ml, were collected at 0.25, 0.5, 1, 2, 4 and 6 hours after the dose administration.

The blood samples were obtained in heparinized syringes. Plasma was separated by centrifugation in microcentrifuge tubes and stored at $-4^{\circ}C$ until analyzed next day. The volunteers were instructed to collect their urine voids, when ever they needed, in the containers provided for this purpose. After the last blood sample at 6 hours they were asked finally to empty their bladders and to submit the voids. The urine voids of each volunteer were cumulated separately in marked one litre round bottom flasks, thoroughly mixed, and then carefully measured and recorded. A 15ml aliquot was frozen for subsequent analysis of acetaminophen and its two major metabolites the next day.

ii) Water deprivation studies: Five days after the control studies, as per direction, volunteers reduced their daily water intake upto 300ml for three days. Afterwards on the fourth day at 7.00 a.m. the volunteers started complete water deprivation

when they took 50ml of simple water and a cup of tea (≈100 ml). The volunteers took their lunch at 2.00 p.m. without any fluid. At the 10th hour of the water deprivation, the volunteers were asked to urinate and empty their bladders. At the 12th hour a 20ml blood sample was taken for the evaluation of physiologic and biochemical effects of water deprivation upto this stage. At the 14th hour, two hours after the blood sample, the volunteers collected their urine voids in the containers provided for this purpose. The mid-point of the intervals of the two urinations (at 10th and 14th hours) coincided with the 12th hour blood sample. Following the urine samples the subjects took their dinner without any fluid. Due to thirst they took comparatively less food than their normal intake of food.

Next day early in the morning on completion of 22 hours' of water deprivation, the volunteers emptied their bladders and then reported to the laboratory at 7.00 a.m. The blood samples for the evaluation of physiologic and biochemical effects of 24 hours of water deprivation were collected. Then a single dose of 25ml of Calpol (600mg acetaminophen) was given to each volunteer and they were advised to take only 50ml of water afterwards. The volunteers were asked to collect their urine voids 2 hours' after the dose (26 hours after the water deprivation) and the urine voids were recorded. A 0.5ml sample of the urine from each void was taken out for the biochemical examination and renal clearance tests, the remaining voids were kept for mixing with

the urine voids collected at the end of the study. The details of blood sampling in this study were the same as described in the control study. No fluid was given until the end of the study.

2.3. ANALYSIS OF ACETAMINOPHEN AND ITS TWO MAJOR METABOLITES IN BLOOD AND URINE

2.3.1. Choice of Assay Method

A number of assay methods for the estimation of acetaminophen and its two major metabolites in biological fluids are available. The spectrophotometric methods[135-138] are time consuming, laborious and less precise. The GLC methods[115,133,139-141] require derivatization and flame ionization detectors or mass spectrometer. On the other hand HPLC methods[142,143] are relatively simple, equally precise and offer high resolution. These methods require no extraction prior to injection onto the column. Only deproteinization of plasma and appropriate dilution of urine samples are required before the injection onto the column. In the present work the recently reported HPLC method[143] was used after slight modification.

2.3.2. HPLC Procedure

An HPLC system comprising a pump attached with a UV variable detector, a data processor and a 50µl injecting port was used for the analysis of the drug and metabolites. A 50µl plasma

sample or 10-50 fold diluted urine sample was mixed with an equal volume of 400µg/ml of theophyline solution in 6% perchloric acid. Theophylline was used as an internal standard while perchloric acid for the deproteinization of plasma proteins. The final volume in each 500µl polypropylene microcentrifuge tubes was 100µl. The content of microcentrifuge tubes were vortexed for 20 seconds and then centrifuged for 10 minutes at 4000 r.p.m. A 5µl portion of the supernatant liquid was injected onto the column (reverse phase, octadecyl C-18, particle size 10µm) using a solvent system comprising acetonitrile/ 0.05 M sodium sulfate (7:93 v/v) adjusted to pH 2.2 with the help of phosphoric acid. The flow-rate was set at 1.5ml/min. to monitor the effluent. Retention times of 3.2, 3.9, 4.5 and 6.4 minutes were obtained for acetaminophen glucuronide, acetaminophen sulfate, acetaminophen and theophylline. A typical chromatogram is shown in Figure-1. Detection was effected at 254 nm.

Both for the urine and the plasma, standard curves for acetaminophen and the two conjugates were prepared in the range from 0.5µg/ml to 25µg/ml. All the six standard curves (three for plasma and three for urine) were linear with a correlation coefficient > 0.99 at 0.001 p level of significance. The intra and inter day variations within two weeks were between 2-7%. For the determination of acetaminophen and its two major metabolites in plasma and urine down to 1µg/ml both the sensitivity and precision of the method were found to be excellent.

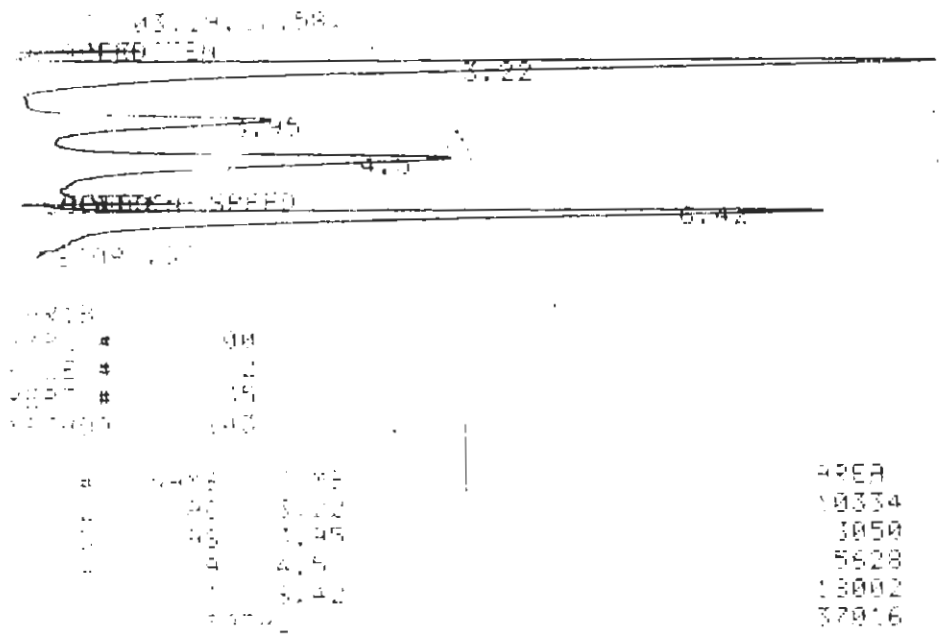


Figure 1 - Report and chromatogram of acetaminophen (A) acetaminophen glucuronide (AG) acetaminophen sulfate (AS) and internal standard (T) in plasma.

2.4. BIOCHEMICAL ANALYSIS

The biochemical analysis of total proteins, enzymes, hemoglobin, chloride ions, lipids, triglycerides, glucose and urea was carried out using the Merckotest diagnostic kits according to the method included in the Merck manual "Directions for Use: Clinical Chemistry".[144] Generally pathonorm L was used as the control sera for the evaluation of accuracy of the analyses.[145]

2.4.1. Protein Analysis

i) Total proteins: The total serum proteins was determined by the Biuret method. To check the precision and accuracy of the method, Pathonorm-L was employed and the results were found to be 100±3% (n=5) of the labelled content.

ii) Electrophoresis: The major groups of serum proteins albumin and α_1, α_2, β and γ globulins - were separated by electrophoresis.[146] Cellulose acetate strips and trisbarbital buffer, pH 8.8 with an ionic strength 0.067, were used for electrophoresis. At the end of the electrophoresis, protein bands were stained with Ponceau-S dye and the strips were destained by three successive washes of 5% acetic acid and then dehydrated by two absolute methanol washes. To facilitate scanning the strips were made transparent with a 4% clearing solution of polyethylene glycol in 30% v/v glacial acetic acid and methanol mixture. Protein bands were scanned on a densitometer using a 575nm filter.

For the quality control, every time while doing the electrophoresis the control sera, seronorm protein, was used.[147] The values determined for the control were always in good agreement with those of the given for control sera.

2.4.2. Blood Acid-Base Status and Gas Analysis

Carbon dioxide and oxygen pressures of the blood, its pH, total carbon dioxide, actual bicarbonate and base excess in heparinized blood were determined on an automated gas analyzer, BME-33 Radiometer, in the National Institute of Cardiovascular Diseases, Karachi, Pakistan. Before use the instrument was duly checked with qualicheck alkalemia, normal and acidemia controls.

2.4.3. Enzymes Analysis

Glutamate oxaloacetate transaminase (GOT) and glutamate Pyruvate transaminase (GPT) in serum were determined by Reitman and Frankle method. Creatine-N-phosphotransferase (CK) was determined by optimized UV method while creatinine in serum and urine was assayed by picrate method. All the enzymes were estimated on an automated biochemical analyzer, Ames pacer Cadit. For the evaluation of the accuracy and precision of these estimations simultaneously the, control sera, Autonorm was also analyzed.[148] The variation of the results of the control was never more than $\pm$5% of the labelled values.

2.4.4. Hematology

i) Hematocrit: The packed cell volume was determined by centrifuging the heparinized blood in capillary tubes on a hematocrit centrifuge for 10 minutes at 5000 r.p.m. The hematocrit value was determined from these capillary tubes by reading them over spiral type hematocrit scale.

ii) Hemoglobin: The hemoglobin content was determined through its cyanidation with potassium cyanide and hexacyanoferrate solution. Hemoglobin cyanide concentration was determined by spectrophotometric method. Merckotest hemoglobin cyanide standard solutions of various concentrations were used for making the standard calibration curve (correlation coefficient = 0.95 at a significance level of 0.001). A fresh calibration curve was prepared whenever hemoglobin was determined. All the curves did not show more than ± 5% coefficient of variation.

iii) Differential count: The differential counting of leukocytes was done by manual chamber counting method.*

2.4.5. Electrolytes Determination

i) Sodium and potassium ions: The content of sodium and potassium ions in blood and urine were determined by flame photometery.[149] The serum samples were diluted with deionised water

*The differential counting was made by a very experienced pathologist.

upto 1,000 times so as to determine the concentration of sodium and potassium ions respectively, whereas the urine samples were diluted upto 10,000 times accordingly for this purpose. The instrument was calibrated prior to the determination by using Pathonorm L.

ii) Chloride ions: The quantitative estimation of chloride ions in serum and urine was done by mercumetric chloride titration using diphenylcarbazone as an indicator. Pathonorum L was used to check the reliability of the determinations.

2.4.6. Lipids, Triglycerides, Glucose and Urea

Lipids and triglycerides in serum were determined respectively by Zollner and Krisch method of sulfophosphovanillin reaction and Witter and Whitner method of enzymatic hydrolysis for formazane formation. Every time of determination Pathonorm L was also analysed as control sera to evaluate the accuracy and precision of the method. The variation in the results of control sera from standard values was never more than $\pm$ 5%.

Plasma levels of glucose and urea were determined by o-toluodine and diacetyl monoxime (DAM) method. For quality control Pathonorm L was analysed alongwith the samples. The results of control was always within $\pm$ 5% of standard values.

2.5. PHARMACOKINETIC COMPUTATION

2.5.1. Disposition Kinetics:

Using the non-compartmental approach[150] the plasma levels and renal excretion of unchanged acetaminophen and its two conjugates were employed to compute their individual disposition kinetics. To calculate the renal clearance and mean residence time of the parent drug and metabolites the following equations were used:

$$CL_R = dxu/dt \; / \; C_t \qquad \text{Eq.1}$$

$$MRT = AUMC_{0-\alpha} / AUC_{0-\alpha} \qquad \text{Eq.2}$$

where;

CL_R = Renal clearance, (ℓ/hr)

dxu/dt = Exertion rate constant, (mg/hr)

C_t = Plasma concentration, (mg/ℓ)

MRT = Mean residence time, (hr)

AUC = Area under the curve, (mg/hr/ℓ)

AUMC = Area under the first moment curve, (mg.hr^2/ℓ)

For computation of above mentioned parameters a computing program for a programable calculator Fx-602 was written. Program listing and details of its flow-and executions are given in Appendix-1. Area under the plasma level curves of metabolites was also determined through this program.

2.5.2. Absorption Kinetics:

The plasma levels of the unchanged drug were employed to compute the absorption rate constant. The plasma levels of acetaminophen were analysed according to the following equations:

$$C = C(O)\ (e^{-\lambda_z t} - e^{-k_a t}) \qquad \text{Eq. 7}$$

where;

C = Drug concentrations in plasma at any time t (mg/).

C(O) = initial (fictive) or back extrapolated post absorptive phase plasma drug concentrations at 0 time, (mg/1).=

λ_z = Dispositions rate constant, (hr^{-1})

k_a = Absorption rate constant, (hr^{-1})

t = time, (hr)

The residual method was employed to calculate absorption rate constant. A pharmacokinetic computing program[151] for a programable calculator Casio-FX 502 was utilized for the fitting and analysis. The half-lives were calculated by the general formula; $\ell n2/k$.

2.6. STATISTICAL ANALYSIS:

All the physiological, biochemical and pharmacokinetic parameters determined in normal and water deprived states were processed statistically. Mean values, $\bar{x}$, and their standard error of mean, SEM, were calculated for each parameter. To evaluate the significance of the difference among the two states, analysis of variance, ANOVA, was performed.[152] For computation of mean, SEM and ANOVA a computing program for a programmable calculater Casio FX-602 was written. Program listing and details of its flow and execution are given in Appendix II.

3. RESULTS AND DISCUSSION

3.1. PHYSIOLOGIC AND BIOCHEMICAL CHANGES

3.1.1. Body Weight

The present study revealed a significant loss ($p \leq 0.05$) in the body weight within 12 hours of the water deprivation. This is in accordance with the results of many other studies in humans[17,18] and animals.[5-15] The minimal time in which significant loss in the body weight occurs may not be checked through the available data as the first observation was taken 12 hours after the water deprivation. However, it seems that the significant loss in body weight (3%) occurs in the 12th hour of water deprivation. This loss in weight is a little more than the loss reported in the minimal time of 8 hours in rats.[15] The observed weights, hematocrit and total serum proteins alongwith the percentage difference and statistical significance are reported in Table II. The weight loss becomes clinically important if it is above 5%.[16] Although the total percent loss in 24 hours is numerically too small to be clinically important, it is still statistically significant ($p \leq 0.01$) because of a consistent loss of weight ranging from 1.54% to 4.12% in all the 10 volunteers. It is also note-worthy that the major fraction of the loss in 24 hours occurred within the first 12 hours of water deprivation, i.e. 2.85% out of a total of 3.17% observed in 24 hours. The 0.29% weight loss during 12 and 24 hours of the deprivation is not statistically significant and hence negligible. It reflects that the weight loss during 24 hours is almost equivalent to the fluid

TABLE II

EFFECT OF WATER DEPRIVATION ON BODY WEIGHT, TOTAL SERUM PROTEINS AND PACKED CELL VOLUME IN HUMANS(n=10)

Parameter	Level (mean ± SEM)			Percent change in mean levels			Mean ± SEM % change in all volunteers		
	0 hr (normal)	12 hr	24 hr	0-12 hr	12-24 hr	0-24 hr	0-12 hr	12-24 hr	0-24 hr
1. Body weight; Kg	64.8±1.09	63.69±1.09	62.75±1.11	-1.71*	-1.48ns	-3.16*	-2.85±0.37	-0.29±0.18	-3.17±0.28
2. Total serum proteins;g/l	83.56±2.72	69.46±4.73	86.41±3.04	-16.87ns	24.40ns	3.41ns	-13.61±7.80	23.06±12.03	4.87±3.15
3. Packed cell volume;H.Ct.%	41.80±1.39	40.00±0.84	47.00±2.21	-4.31ns	17.50*	12.44*	-4.05±2.38	14.48±5.12	9.46±3.24

* = Significantly different at $p \leq 0.05$.

ns = No significant difference at $p \leq 0.05$.

output of the body. During 24 hours of the deprivation the water intake was almost negligible except that included in food ≈ 100ml. The initial urine output was normal. If the water loss through perspiration and respiration is also taken into account, it would appear that the total loss during 24 hours' deprivation is mainly due to the water output of the body as well as due to a comparatively less consumption of food because of the thirst. The catabolism of body fats and protein does not seems to be the reasons of weight loss in any proportion for this period of water deprivation.

3.1.2. Acid-Base Status of Blood

Significant increases in the actual bicarbonate content, the base excess and total carbon dioxide were observed after 12 and 24 hours of water deprivation. Although the increase in these three parameters was gradual throughout the deprivation, the bicarbonate and total carbon dioxide values did not show any significant increase uptil 12 hours, the base excess was found to be significantly increased ($p \leq 0.05$) even after 12 hours. The results of blood gas analysis and acid-base status in normal condition and after 12 and 24 hours of water deprivation are given in Table III alongwith the percent changes occurred during 0-12, 12-24 and 0-24 hours. An overall view of the changes in acid-base status and blood gases may be seen in Figure-2. Besides the above mentioned significant increases, an increasing tendency

TABLE III

EFFECT OF WATER DEPRIVATION ON BLOOD (VENOUS) GASES AND ACID-BASE STATUS IN HUMANS (n=10)

Parameter	Level (mean ± SEM)			Percent change in mean levels			Mean ± SEM % change in all volunteers		
	0 hr (normal)	12 hr	24 hr	0–12 hr	12–24 hr	0–24 hr	0–12 hr	12–24 hr	0–24 hr
1. Actual blood bicarbonate; mmol/l	21.07±0.90	21.72±0.77	22.56±0.84	3.08^{ns}	3.87^{ns}	7.07^{*}	2.74±2.95	6.95±4.34	11.11±4.07
2. Carbon dioxide pressure; mm Hg.	41.24±2.64	43.36±1.67	46.10±2.28	5.14^{ns}	6.32^{ns}	11.78^{ns}	5.76±7.62	8.66±6.75	16.94±9.16
3. Oxygen pressure;mm Hg	32.88±3.25	33.44±3.73	26.29±1.91	1.70^{ns}	-21.38^{ns}	-23.08^{ns}	12.58±12.02	-10.92±10.68	-4.67±11.42
4. Blood pH	7.33±0.02	7.31±0.01	7.31±0.01	-0.27^{ns}	0.00^{ns}	-0.27^{ns}	0.04±0.15	0.07±0.24	0.07±0.34
5. Blood base excess;mmol/l	-4.33±0.68	-3.92±0.68	-3.00±0.48	-9.97^{ns}	-23.47^{*}	-30.72^{*}	14.25±24.98	21.72±8.75	32.38±5.37
6. Blood total carbon dioxide;	22.32±0.97	22.83±0.80	23.99±0.93	2.28^{ns}	5.08^{ns}	7.48^{*}	1.96±2.40	8.24±2.86	11.23±4.05

* = Significantly different at $p \leq 0.05$.

ns = No significant difference at $p \leq 0.05$.

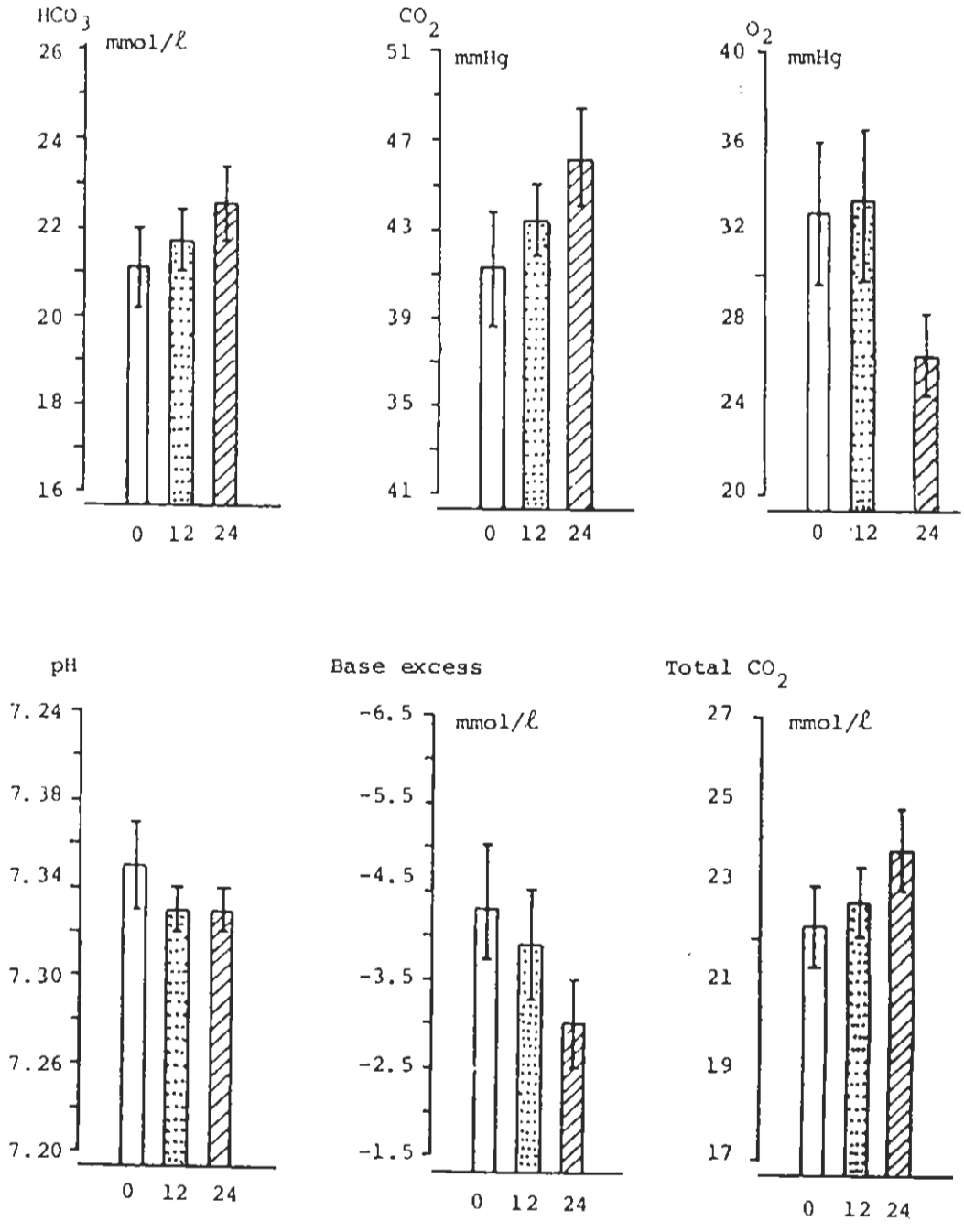


Figure 2 - A comparison of blood gases and acid-base status in normal and water deprived (12 and 24 hours) states in humans (n=10).

(though statistically insignificant) in PCO_2 was noted throughout the deprivation period, whereas a decreasing tendency (statistically insignificant) in PO_2 was noted after 12 hours' deprivation. These results seem to predict an overall situation resembling metabolic alkalaemia and are in good agreement with those reported for contraction alkalosis in water depleted edematous patients.[58] However, these results are in contradiction to those of the previous data on the water deprived rats, which showed no significant change in acid-base status. This difference may be accounted for on the basis of the physiologic and biochemical variations in the body systems of both humans and rats. In view of our observations, a decrease in the bicarbonate concentration,[56,57] may be due to the unaltered lipid metabolism in the present subjects compared with that recorded at an increased rate in the humans.[57] With the available data it is difficult to visualize whether HCO_3^- is a primary variable or PCO_2. However, a more consistent and profound increase in HCO_3^- in comparison to that of PCO_2 suggests that HCO_3^- is probably the primary variable. The results(Table III) indicate an originally reduced H^+ ion concentration (i.e. increase in basicity) which is immediately compensated by the blood buffering system or renal tubular mechanism. The H^+ ion loss from the blood may be explained in terms of potassium depletion as observed during the deprivation (section 3.1.8). When H^+ ions move into the cells to replace the potassium ions, relative increase in HCO_3^- ions

occurs because of the hydrogen ion depletion in the extracellular fluid and plasma, the increased pH is compensated by the buffering system, although an over-all increase in bicarbonate content, base excess and PCO_2 occurs, a situation observed in metabolic alkalaemia. It could be speculated that the slight change observed in 24 hours of water deprivation may become more profound in severe dehydration and thus ultimately affect the pharmacokinetic processes which in turn at times depend upon the acid-base status of the body fluids.

3.1.3. Serum Proteins

No significant change ($p \leq 0.05$) has been found in the total serum proteins levels in 24 hours of water deprivation. The total and individual protein levels in the normal and water deprived states are given in Table IV. The changes in serum protein during 24 hours' water deprivation may be seen in Figure-3. The total serum proteins in normal condition and on 24 hours' deprivation are 83.56 ± 2.72 g/ℓ and 86.41 ± 3.04 g/ℓ respectively and do not show any significant increase as noticed in the animals.[5,9,11,15,21] It may be explained on the basis of the small period i.e. 24 hours, for which the humans subjects in the present study were water deprived. However, it is noteworthy that 7 volunteers out of 10 showed an increase in the total protein level. From a small increase (4.87%) in serum protein level a proportional/equal decrease in plasma volume may be expected as

TABLE IV

EFFECT OF WATER DEPRIVATION ON SERUM LEVELS OF ALBUMIN AND GLOBULINS AND THEIR RATIO IN HUMANS (n=10)

Parameter	Level (mean ± SEM)			Percent change in mean levels			Mean ± SEM % change in all volunteers		
	0 hr (normal)	12 hr	24 hr	0-12 hr	12-24 hr	0-24 hr	0-12 hr	12-24 hr	0-24 hr
1. Serum albumin content; g/l	42.27±1.71	41.55±2.66	40.23±2.46	-1.70^{ns}	-3.18^{ns}	-4.83^{ns}	-o.51±6.07	-4.60±4.78	-4.71±4.92
2. Serum globulin content; g/l	38.24±3.71	32.65±3.22	46.64±3.50	-14.62^{ns}	42.85*	21.97*	-4.84±11.59	38.40±13.83	23.92±7.07
3. Serum albumin/globulin ratio	1.16±0.12	1.30±0.12	0.89±0.10	12.07^{ns}	-31.54*	-23.28*	8.24±9.12	-28.17±7.02	-21.44±7.00
4. Serum α_1 globulin content; g/l	1.87±0.17	1.32±0.26	2.08±0.14	-29.91^{ns}	57.56^{ns}	11.23^{ns}	-21.75±15.32	82.35±45.49	13.26±8.34
5. Serum α_2 globulin content; g/l	6.99±0.71	6.30±0.61	9.50±0.61	-9.87^{ns}	50.79*	35.91*	2.86±22.73	45.21±9.73	38.81±18.02
6. Serum β globulin content; g/l	8.35±1.59	9.49±0.36	14.59±1.49	13.65*	53.79*	74.73*	43.13±13.76	40.40±10.92	88.74±18.39
7. Serum γ globulin content; g/l	21.02±2.77	15.54±2.66	20.81±2.80	-26.07^{ns}	33.91^{ns}	-1.00^{ns}	-4.41±15.18	35.01±18.34	5.59±11.58

* = Significantly different at $p \leq 0.05$.

ns = No significant difference at $p \leq 0.05$.

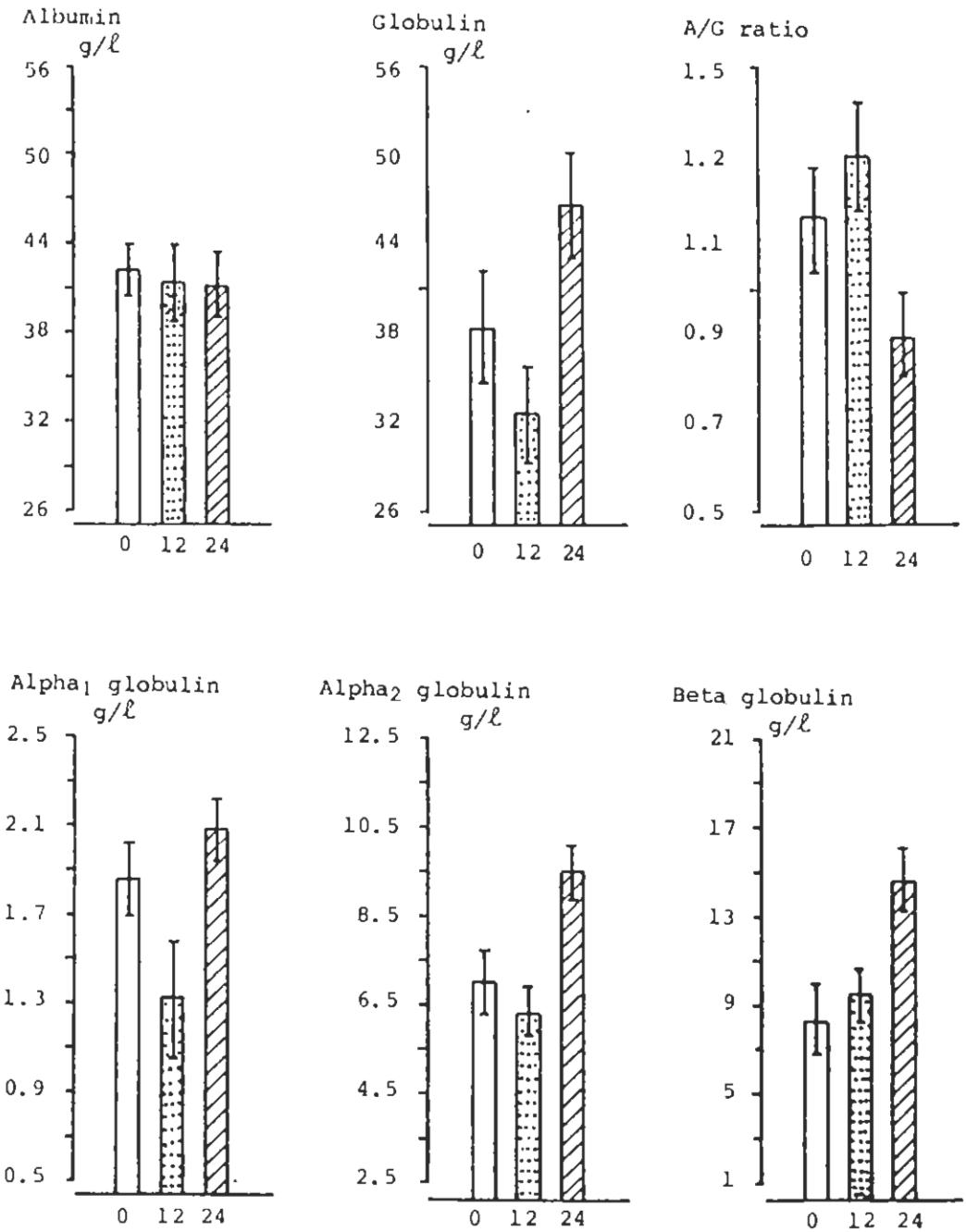


Figure 3 - A comparison of serum levels of albumin and globulins and their ratio in normal and water deprived states (12 and 24 hours) in humans (n=10).

the changes in serum protein have always been associated with the change in plasma volume in animals.[9,11,21] Although no statistically significant ($p \leq 0.05$) change found in the albumim levels in normal and water deprived states, a small but consistent decrease in albumim fraction of 4 volunteers out of 6 was noted. After 24 hours of water deprivation this decrease might have been more profound. The decrease in albumim may be explained in terms of increased catabolism or decreased synthesis of albumim in the liver due to the stress of water deprivation. No comparable data on the effects of water deprivation on individual proteins is available except a study which mentioned an increase in the albumim levels in rats after thermal dehydration. This difference may be attributed to the variations in the in-vivo mechanism of humans and rats and to the nature of induction of dehydration.

Unlike the albumim, a significant change ($p \leq 0.05$) has been noted in the serum globulim contents. The serum globulin is considerably increased ($38.4 \pm 13.83\%$) during 12 to 24 hours of water deprivation. The increase in globulin has no effect on the total proteins because globulin is about 50% of the total proteins and the increase in globulin is compensated by a decrease in albumim. As a consequence of significant increase in globulin content the A/G ratio is significantly decreased ($p \leq 0.05$) in 24 hours from 1.16 ± 0.12 to 0.89 ± 0.10 which indicates some

disturbed function of liver and kidney during water deprivation. No appreciable change has been found in α_1 & γ globulin content although, significant increase in α_2 and β globulin resulted. The increase in β globulin content just after 12 hours was more than 40% and it reached almost 90% after 24 hours (Table IV). The α_2 globulin showed a significant increase (45.21±9.73%) between 12 and 24 hours giving a net increase of 38.81±18.02% at 24 hours. The increase in α_2 and β globulins may arise from increases in heptoglobin and transferrin respectively. Hepatoglobin binds with hemoglobin released after the red blood cells are destroyed, whereas transferrin is responsible for the transport of iron in the plasma. The plasma level of transferrin is increased in humans with iron deficiency. This seems to be reasonable as some sort of deficiency/destruction of red blood cells occurs in water deprivation[20,34] alternately the increased level of α_2 and the β globulin may be explained in terms of some disfunctioning of liver and kidney.

3.1.4. Hematocrit

It is well known that water deprivation leads to an increase in hematocrit which in the present case remained almost unchanged (41.8±1.39 vs 40.00±0.84) during the first 12 hours. However, a consistent and statistically significant ($p \leq 0.05$) increase of about 15% was observed between 12 and 24 hours of water deprivation, which resulted in a net significant ($p \leq 0.05$)

increase of ≈ 10% in hematocrit value during 0-24 hours (Table II). An apparent relationship between average increase in hematocrit (9.46±3.24%) and the total serum proteins (4.87±3.15%) seems to predict about 5% loss in plasma volume due to water deprivation for 24 hours. This is obvious from the percentage increase in hematocrit which is two fold compared with the decrease in plasma volume.

It is interesting to note that the total body water constitutes 60% of the total body weight. On the basis of a decrease in plasma volume a loss of 5% could be expected in the whole body water and thus the total net loss in body weight would be 3%, whereas the observed average body weight loss value is 3.17%. It may be concluded that in the initial stage (24 hours) of water deprivation, all water compartments i.e. extracellular fluid, intracellular fluid and plasma volume reduce equally and no preference to save one compartment at the expense of the other is noticed. This finding is contrary to the observations made in animals.[5,6,20]

3.1.5. Hemoglobin and Differential Count

No statistically significant ($P \leq 0.05$) change has been observed in the values of hemoglobin content and differential count of all leukocytes after 12 hours' of water deprivation. However, between 12 to 24 hours a consistent and statistically

significant ($p \leq 0.05$) increase in hemoglobin content, neutrophil and eosinophil counts was noted (Table V). On the other hand no change was observed in lymphocyte and monocyte counts in this period. Since no such data are available in the literature, these results cannot be compared.

The stress and hemoconcentration seems to be the governing factors for these alterations. The average percent increase found in hemoglobin concentration ($5.97 \pm 2.96\%$) after 24 hours is comparable with the percent increase in the serum protein content ($4.89 \pm 3.15\%$). This further supports the prediction of about 5% loss in the plasma volume. It is noteworthy that all the significant changes in hematocrit, total proteins, hemoglobin and different globulins occur between 12 and 24 hours of water deprivation. This is evident from the fact that hemoconcentration manifests itself after 12 hours of water deprivation. During 12 and 24 hours of water deprivation an increase in neutrophils, may be explained in terms of stress of water deprivation as well as hemoconcentration. The decrease of lymphocytes during this period may be accounted for by the relative reduction due to increase in the percentage of neutrophils and probably in terms of stress.

3.1.6. Enzymes

Glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) creatine-N-phosphotransferase (CK) have

TABLE V

EFFECT OF WATER DEPRIVATION ON HEMOGOBIN CONTENT AND DIFFERENTIAL COUNT OF LEUKOCYTES IN HUMANS (n=10)

Parameter	Level (mean ± SEM)			Percent change in mean levels			Mean ± SEM % change in all volunteers		
	0 hr (normal)	12 hr	24 hr	0-12 hr	12-24 hr	0-24 hr	0-12 hr	12-24 hr	0-24 hr
1. Hemoglobin content; mmol/l	7.57±0.29	6.68±0.22	7.70±0.54	1.45^{ns}	0.26^{*}	1.27^{ns}	1.08±3.67	7.33±2.02	5.97±2.96
2. Neutrophils count; %	73.00±4.80	55.40±2.00	71.20±2.30	-24.11^{ns}	28.52^{*}	-2.47^{ns}	-9.72±5.56	9.68±2.84	0.84±4.41
3. Lymphocytes count; %	19.25±3.77	29.20±1.69	22.60±1.72	51.69^{ns}	-22.60^{*}	17.40^{ns}	66.98±24.66	-22.77±3.15	20.34±15.14
4. Eosinphils count; %	3.50±1.26	1.80±0.37	2.60±0.40	-48.57^{ns}	44.44^{*}	-25.71^{ns}	-30.95±11.74	55.67±19.44	-5.95±32.67
5. Monocytes count; %	4.25±0.48	3.60±0.40	3.20±0.73	-15.29^{ns}	-11.11^{ns}	-24.71^{ns}	-10.00±10.00	-2.00±28.36	-35.83±13.15

* = Significantly different at $p \leq 0.05$.

ns = No significant difference at $p \leq 0.05$.

been monitored to evaluate the effect of dehydration on the release of these enzymes. The observed levels of these enzymes and the percent differences occurred in the water deprived conditions are given in Table VI. After 12 hours a considerable decrease (≈ 43%) in CK levels (98.2±17.64 vs 63.16±8.94) was observed. Between 12 and 24 hours no further decrease was noted. It could therefore, be concluded that due to the stress produced by thirst the mobility and activity of the volunteers were reduced thus resulting in a significant decrease in the levels of CK. The same may be true for GOT and GPT. No significant change has been observed in GPT and GOT (Table VI). Both the enzymes showed a very small decrease in concentrations during water deprivation. This may arise from lack of mobility and activity of thirsty volunteers. The percent decrease in the case of GPT was more uniform and consistent than that of GOT. It bears a statistical significance at $p \leq 0.05$, although it has no clinical importance. The enzyme levels obtained in the present work indicate no liver disorder or any other change of clinical significance.

3.1.7. Renal Physiology

Since the functioning of kidney greatly depends upon the glomerular filteration rate, the blood flow to the kidney and thence the resulting filteration fraction are of indispensible importance in the evaluation of the effects of dehydration on the renal physiology and drug disposition kinetics. Therefore, in the present investigation urine flow-rate, serum creatinine levels

TABLE VI

EFFECT OF WATER DEPRIVATION ON SERUM LEVELS OF ENZYMES: GOT,GPT AND CPK IN HUMANS (n=6)

Parameter	Level (mean ± SEM)			Percent change in mean levels			Mean ± SEM % change in all volunteers		
	0 hr (normal)	12 hr	24 hr	0-12 hr	12-24 hr	0-24 hr	0-12 hr	12-24 hr	0-24 hr
1. GOT (ASAT); U/l	26.20±3.80	28.00±2.19	24.00±1.93	6.87^{ns}	-14.29^{ns}	-8.40^{*}	15.39±17.81	-15.08±7.98	-14.12±5.26
2. GPT (ALAT); U/l	26.60±4.90	30.20±5.11	25.16±3.51	13.53^{ns}	-16.69^{ns}	-5.91^{ns}	19.15±11.39	-16.40±11.23	-4.66±8.01
3. CPK; U/l	98.20±17.64	56.00±7.33	63.16±8.94	-42.97^{*}	12.79^{ns}	-35.68^{*}	-39.35±8.19	6.96±10.46	-35.01±11.83

* = Significantly different at $p \leq 0.05$.

ns = No significant difference at $p \leq 0.05$.

and glomerular filteration rate were monitored to observe the effects of dehydration on the function of kidney. The observed values are given in Table VII. The urine flow-rate in the first 12 hours of the deprivation reduced to < 0.5ml/min from its initial value ≈ 1ml/min. However, due to the much variability among the flow-rates of individual volunteers this big difference was not statistically significant. In the next 12 hours of the deprivation the urine flow decreased upto < 0.3ml/min, which is the minimal possible rate to excrete out about 600 mosmol/day (estimated from electrolytes and urea content of urine) with the maximum possible urine osmolality, 1400 mosmol/kg. The decrease in the urine flow-rate during 12 to 24 hours as well as from 0 (normal condition) to 24 hours is significant at $p \leq 0.05$ level.

This finding is consistent with previous works reported for humans[17,18] and animals.[54,55] Besides the maximum activity of antidiuretic hormone (ADH) which takes place during the thirst, the minimum possible urine formation is also due to reduced glomerular filteration rate (GFR) as revealed from the observed creatinine clearance values in the water deprivation (Table VII). The volunteers showed a significant decrease ≈ 57% in their GFR (creatinine clearance value) after 24 hours of the water deprivtion. The decrease in urine flow-rate and GFR exhibit the severity of dehydration-effects on renal physiology, which in turn might affect the disposition of those drugs which depends upon

TABLE VII

EFFECT OF WATER DEPRIVATION ON URINE FLOW RATE SERUM LEVEL OF CREATININE AND ITS RENAL CLEARANCE IN HUMANS

Parameter	Level (mean ± SEM)			Percent change in mean levels			Mean ± SEM % change in all volunteers		
	0 hr (normal)	12 hr	24 hr	0-12 hr	12-24 hr	0-24 hr	0-12 hr	12-24 hr	0-24 hr
1. Urine flow rate; ml/hr(n=10)	58.00±20.41	24.94±2.32	17.16±1.38	-57.00^{ns}	-31.19*	-70.41*	-32.91±14.38	-34.08±5.28	-51.76±8.73
2. Serum creatinine; µmol/l(n=10)	91.31±4.99	85.77±9.49	101.07±7.69	-6.07^{ns}	17.89^{ns}	10.69^{ns}	-5.43±8.86	10.56±12.46	5.86±7.35
3. Creatinine renal clearance; l/hr (n=6)	7.68±0.34	6.37±0.56	5.16±0.14	-17.06^{ns}	-19.00^{ns}	-32.81*	-15.59±9.95	-16.12±8.60	-32.35±3.34

* = Significantly different at p ≤0.05.

ns = No significant difference at p≤ 0.05.

glomerular filteration and urine flow-rate for their excretion. The possible reasons of reduced GFR may be suggested in terms of reduced renal blood flow and hemoconcentration.

3.1.8. Serum Electrolytes and their Renal Clearance

Electrolytes, particularly sodium and chloride ions are the main osmotically active solutes of extracellular fluid (ECF). Since the body fluid is regulated by osmoreceptors, the regulation in the sodium content regulates ECF and thus it eventually maintains the total body fluid. The sodium content regulation is done by the kidney through its renal excretion or retention as the situation requires. On the other hand potassium is responsible to regulate the osmolality of intracellular fluid. The study of changes in the levels of these electrolytes and their renal clearance is of primary importance in evaluating any change in the body fluid compartments as a consequence of water deprivation. The determined serum levels and the renal clearance values of electrolytes are reported in Table VIII. A significant increase in sodium ions was noted after 12 and 24 hours of the water deprivation. The increase in sodium ion indicates the activity on the part of osmoreceptors to increase ADH release. This increase in ADH release retains the maximum water to combat with water deprivation for the preservance of body water. The estimated average serum osmolality values were found to be 291, 306 and 320 mosmol/kg respectively at O(normal), 12 and 24 hours of water deprivation. It is evident that even after 12

TABLE VIII

EFFECT OF WATER DEPRIVATION ON SERUM LEVELS AND RENAL CLEARANCES OF ELECTROLYTES IN HUMANS (n=10)

Parameter	Level (mean ± SEM)			Percent change in mean levels			Mean ± SEM % change in all volunteers		
	0 hr (normal)	12 hr	24 hr	0-12 hr	12-24 hr	0-24 hr	0-12 hr	12-24 hr	0-24 hr
1. Serum chloride; mmol/l	113.70±2.83	104.66±3.35	108.55±2.63	-7.95*	3.79ns	-4.54ns	-8.22±1.83	3.99±2.75	-4.45±2.54
2. Serum sodium; mmol/l	141.60±1.46	148.00±1.67	155.60±2.14	4.52*	5.14ns	9.89*	4.56±1.48	5.22±2.20	9.95±2.08
3. Serum potassium; mmol/l	6.40±0.64	4.66±0.40	4.92±0.27	-27.19*	5.58ns	-23.13*	-25.71±6.70	3.76±7.18	-23.93±5.92
4. Chloride renal clearance;	0.82±0.15	0.54±0.64	0.79±0.14	-34.15ns	46.30*	-3.66ns	-21.16±21.76	55.99±20.22	-11.67±23.00
5. Potassium renal clearance; ml/hr	0.47±0.09	0.52±0.06	0.66±0.06	10.64ns	26.92*	40.43*	20.20±10.72	36.52±9.10	63.98±17.37
6. Sodium renal clearance; ml/hr	17.06±5.17	47.55±12.76	77.62±11.17	178.72ns	63.24*	354.98*	234.30±92.25	108.98±46.38	517.4±134.95

* = Significantly different at $p \leq 0.05$.

ns = No significant difference at $p \leq 0.05$.

hours the increment on maximum osmolality value, =290m osmol/kg, was significant and after 24 hours it became more pronounced. The hyperosmolality where brings about the physiological changes and stress might be responsible for delayed absorption of drug molecules from gastrointestinal tract and other absorption sites. The increase in sodium content is in good agreement with the previous work on humans.[4] However, in that study food deprivation was also accompanying with the water deprivation. The results are also consistent with significant increase in the serum sodium content and the serum osmolality during the water deprivation in rats[20,48] dogs[30] and horses.[10]

Besides the above mentioned sodium retention phenomenon significant increase in renal clearance of sodium was also recorded (Table VIII). It reveals that the retention phenomenon with progressive dehydration reaches the saturation limits and thus excretion of extra sodium starts in response to reduce body water. The urine osmolality after 24 hours of the deprivation was found to be 1470 mosmol/kg It suggests that urine production after 24 hours of the deprivation was at its maximum limit, because the maximal urine osmolality, =1400 mosmol/kg, has been achieved.

The chloride ions serum content and renal clearance values showed some significant numerical changes (Table VIII) but remained within standard range for the normal humans. Thus the

observed changes do not seem to be of any clinical or physiological significance. Potassium ions level in serum showed no significant changes throughout the deprivation. It is in good agreement with the previous results which were determined after the water and food deprivation for 24 hours in humans.[4] However, a significant increase in renal clearance of potassium was noted in the present work during 12 and 24 hours of the deprivation, indicating a fluid loss in the intracellular compartment.

3.1.9. Glucose, Urea, Triglycerides and Total Lipids

The estimated values of glucose, urea, triglycerides and total lipids at 0 (normal), 12 and 24 hours after water deprivation are given in Table IX. A study of the present results reveals that, despite some numerically significant changes, none of the determined values is out of the standard range for normal healthy humans. Thus from the physiological and clinical point of view water deprivation, at least of 24 hours duration, does not affect glucose, urea, triglyceride and total lipid levels. These results may be compared with only one similar study[4] which included the food deprivation as well on humans. Contrary to the reported significant decrease in glucose and triglyceride levels an insignificant increase was found in the present study (Table IX). This anomaly may be due to the accompanied food deprivation in the work referred to, whereas the volunteers in the present study received food as normally they took. Some significant changes, not clinically or physiologically important, in

TABLE IX

EFFECT OF WATER DEPRIVATION ON SERUM /BLOOD LEVELS OF GLUCOSE, UREA, TRIGLYCERIDES AND TOTAL LIPIDS IN HUMANS (n=10)

Parameter	Level (mean ± SEM)			Percent change in mean levels			Mean ± SEM % change in all volunteers		
	0 hr (normal)	12 hr	24 hr	0-12 hr	12-24 hr	0-24 hr	0-12 hr	12-24 hr	0-24 hr
1. Blood glucose;mmol/l	4.32±0.19	5.24±0.36	5.07±0.27	21.30*	-3.24ns	17.36ns	17.74±7.69	0.21±11.17	18.23±8.19
2. Serum urea; mmol/l	8.64±1.38	5.57±0.90	5.48±0.40	-35.53ns	-1.62ns	-36.57*	-23.46±15.03	8.35±17.25	-25.26±9.67
3. Triglycerides; mmol/l	1.48±0.19	1.29±0.20	1.65±0.21	-12.84*	27.91*	11.99ns	-21.77±7.68	47.04±20.54	11.11±12.69
4. Total lipids; g/l	6.11±0.55	5.12±0.47	5.38±0.46	-16.20ns	5.08ns	-11.95ns	-12.89±11.73	7.07±5.57	-4.99±11.29

* = Significantly different at $p \leq 0.05$.

ns = No significant difference at $p \leq 0.05$.

glucose and triglycerides contents during 0 to 12 and 12 to 24 hours may be accounted for timely variations due to food intake.

The total lipid contents in the present work did not show any numerically or clinically significant change during the 24 hours of water deprivation (Table IX). No comparable data on lipids are available in the literature. Significant decrease in blood urea levels after 24 hours of the deprivation was noted (Table IX). However, the urea level after 24 hours was well within the standard normal range for humans. This decrease may be accounted for comparatively little consumption of food in the state of water deprivation due to the thirst. The results are in general agreement with the reported results.[4]

3.2 EFFECT OF DEHYDRATION ON PHARMACOKINETICS OF ACETAMINOPHEN IN HUMANS

3.2.1. Plasma Levels

The mean plasma levels of acetaminophen (unchanged) in the normal and in the water deprived subjects are given in Table-X. Time course of plasma levels in both the states is shown in Figure 4. Significant difference ($p \leq 0.05$) in plasma levels were found at sampling times of 0.25 and 6 hours. At 0.25 hr the decline in plasma levels in the state of dehydration (2.66 $\pm$ 0.97 mg/ℓ v/s 5.25 $\pm$ 0.79 mg/ℓ) reflects slower absorption. On the other hand at six hours significantly low plasma levels (1.45 $\pm$ 0.27 mg/ℓ v/s 0.48 $\pm$ 0.19 mg/ℓ) reveal increased disposition. The slow absorption and increased disposition are also indicated by low plasma levels at 0.5 and 4.0 hr after administration of the drug. The lower levels of acetaminophen in the last portion of the profile can be accounted for either due to faster metabolism in dehydration or due to its own accelerated renal clearance. The second possibility cannot be accepted as the analysis of variance does not reveal any significant change at $p \leq 0.05$ in the renal clearance of acetaminophen during water deprivation (Table-XI).

The plasma level versus time profile of acetaminophen in humans reflects the gradual increase and then progressive decline of the plasma concentrations by apparent

TABLE X

PLASMA LEVELS OF ACETAMINOPHEN IN NORMAL AND WATER DEPRIVED HUMANS (n=6)AFTER ORAL ADMINISTRATION OF A SINGLE DOSE OF 600 mg ACETAMINOPHEN

Time (hours)	Plasma levels (mean±SEM),ug/ml		Significant* difference
	Normal	Water deprived	
0.25	5.25 ± 0.79	2.66 ± 0.97	$p \leq 0.05$
0.5	6.63 ± 0.63	6.28 ± 1.21	ns
1	7.39 ± 0.94	7.57 ± 0.84	ns
2	4.57 ± 0.84	5.80 ± 0.30	ns
4	2.68 ± 0.21	2.02 ± 0.31	ns
6	1.45 ± 0.27	0.48 ± 0.19	$p \leq 0.05$

* Evaluated through 7-test.

ns = No significance at $p \leq 0.05$

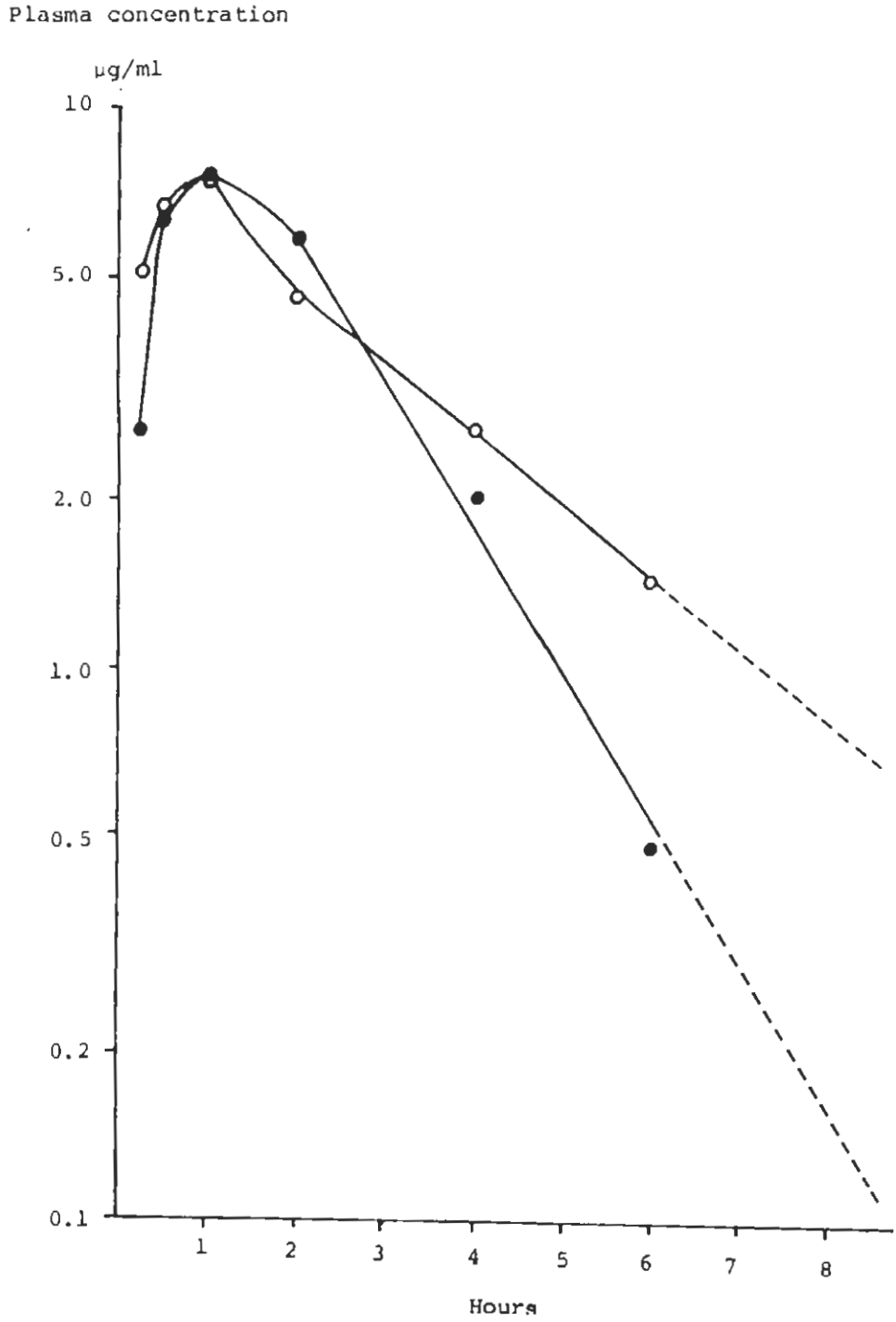


Figure 4 - Mean plasma level versus time profiles of acetaminophen in normal and water deprived (24 hours) states of humans (n=6). Key: o-- Normal ●—Water deprived.

TABLE XI

RENAL CLEARANCE OF ACETAMINOPHEN IN NORMAL AND WATER DEPRIVED SUBJECTS

Volunteer	Renal clearance (ml/hr/kg)	
	Normal	Water deprived
1	38.57	54.65
2	43.37	28.18
3	31.68	27.49
4	35.86	25.41
5	21.43	26.49
6	38.76	25.14
Mean ± SEM	34.95 ± 3.12	31.23±4.71

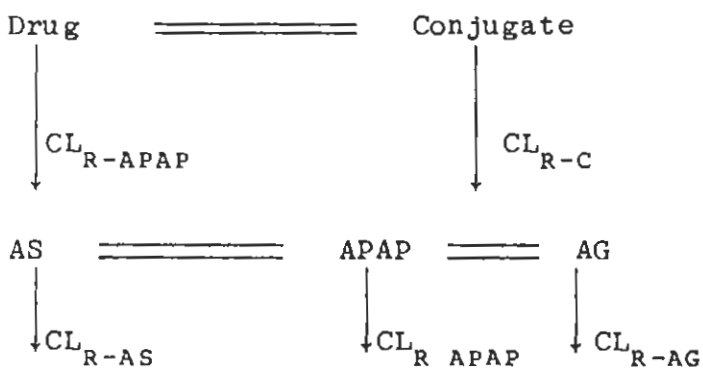
ANALYSIS OF VARIANCE

S.O.V.	S.S.	D.F.	M.S.	F
Treatment (dehydration)	41.780	1	41.780	-
Residual	958.0427	10	95.8043	0.4329^{ns}
Total	999.8227	11	-	-

ns No significant difference at p - 0.05

first order kinetics (Figure 4). A smooth decline in post absorptive phase also depicts a one compartmental model behaviour.

The plasma levels of APAP after peak level at 1.0 hour after the dose in the normal state fall down sharply and then after 2.0 hours disposition seems to be relatively slow. This suggests that the deconjugation contributes to the plasma concentrations. Such deconjugation reactions have been recognised for sulfonamides (deacetylation)[153] & benzodiazepines (deglucuronidation)[154]. The situation may be represented as follows:



3.2.2. Absorption

Absorption kinetics was determined through one compartmental model by applying the feathering technique, as pointed out in Section 3.2.1., the time course of plasma levels depicts one compartment model behaviour. The goodness of fit was determined by evaluating the differences between the

observed plasma levels and the generated levels obtained from the kinetic parameters of one compartment model.

The pharmacokinetic parameters of acetaminophen determined in terms of the one compartment model in both the states are reported in Table-XII. Analysis of variance shows no significant difference in absorption rate constant and absorption half-life. However, in the initial phase (nearly upto 20 minutes) there is a definite trend of slow absorption as revealed by the significant decrease ($p \leq 0.05$) in blood level at first sampling time, i.e., 15 minutes after administration of the drug during dehydration. The dissolution of acetaminophen does not seem to be the rate limiting factor in absorption. That is because of the rapid and complete solubilization of acetaminophen in gastric medium (acidic pH) which might not be affected by the probable less gastric fluid volume due to dehydration. Due to low body water level in dehydration the higher osmolality of blood may interfere with further absorption of any solute. Another factor responsible for the slow absorption may be the low concentration gradient at the absorption site. Higher hematocrit values (low plasma water content) seem to be responsible for instantaneous higher plasma concentration than those in normal state at the site of absorption, which reduce the concentration gradient responsible for passive absorption. Although the absorption

TABLE XII

PHARMACOKINETICS (Mean ± SEM) OF ACETAMINOPHEN IN NORMAL AND WATER DEPRIVED SUBJECTS (n=6)

Parameters	Normal Mean ± SEM	Water deprived Mean ± SEM
$K\ (hr^{-1})$	0.327 ± 0.041	$0.497 \pm 0.054^{+}$
$t_{0.5}(hr)$	2.250 ± 0.230	$1.490 \pm 0.180^{+}$
$K_a(hr^{-1})$	3.803 ± 0.654	3.112 ± 0.540^{ns}
$t_{a0.5}(min)$	$12.530 + 1.950$	$15.620 + 2.750^{ns}$

ns = No significant difference at $p \leq 0.05$

+ = Significant difference at p - 0.05

process is slow in the very initial period of absorption phase, there does not exist an overall change in the absorption half-life as indicated by the analysis of variance. Similarly, neither any significant delay in peak time nor any change in peak level could be observed (Tables-XIII and XIV).

3.2.3. Disposition of Acetaminophen

It has been well documented that the disposition kinetics of acetaminophen follows an apparent first order kinetics. The plasma levels in both the conditions observed in the present study attest to the first order kinetic disposition (Figure 4). The plasma level versus time profilesin the post-absorptive phases of all the subjects were found to have high values of correlation coefficient (> 0.9). This confirms the linear disposition of acetaminophen for the dose administered and supports the one compartmental behaviour or no delayed distribution. The rate constant for disposition (K_{el}) was found to be significantly increased (0.327 ± 0.041 hr^{-1} v/s 0.497 ± 0.059 hr^{-1}) at $p \leq 0.05$. Accordingly a significicant ($p \leq 0.05$) decrease in biological half-life was noted (2.25 ± 0.23 hr^{-1} v/s 1.49 ± 0.18 hr^{-1}). It is noteworthy that all of the six subjects showed this rapid disposition in the state of dehydration. The noncompartmental approach was also applied to evaluate the disposition kinetics. No significant change in the total area under the curve, $AUC_{0-\alpha}$, was found in the water deprivation (0.43 ± 0.05 mg.hr/ℓ v/s 0.36 ± 0.03 mg.hr/ℓ).

TABLE XIII

TIME OF THE MAXIMUM PLASMA CONCENTRATION OF ACETAMINOPHEN IN NORMAL AND WATER DEPRIVED SUBJECTS

Volunteer	Time of maximum concentration (hr)	
	Normal	Water deprived
1	1.0	2.0
2	1.0	2.0
3	1.0	0.5
4	1.0	0.5
5	1.0	1.0
6	0.5	0.5
Mean ± SEM	0.92±0.08	1.08±0.3

ANALYSIS OF VARIANCE

S.O.V.	S.S.	D.F.	M.S.	F
Treatment (dehydration)	0.0833	1	0.0833	-
Residual	2.9167	10	0.2917	0.2857^{ns}
Total	3.0000	11	-	-

ns=No significant difference at p - 0.05

TABLE XIV

MAXIMUM PLASMA CONCENTRATION OF ACETAMINOPHEN IN NORMAL AND WATER DEPRIVED SUBJECTS

Volunteer	Maximum concentration (µg/ml)	
	Normal	Water deprived
1	5.21	5.94
2	6.53	5.82
3	10.90	8.43
4	7.68	8.19
5	9.04	10.50
6	8.56	9.94
Mean ± SEM	7.99±0.81	8.14±0.80

ANALYSIS OF VARIANCE

S.O.V.	S.S.	D.F.	M.S.	F
Treatment (dehydration)	0.0675	1	0.0675	-
Residual	38.9701	10	3.8970	0.0173 ns
Total	-	11	-	-

ns =No significant difference at p - 0.05

However, at $p \leq 0.05$ significantly lower area under the first moment curve, $AUMC_{0-\alpha}$, was observed (1.52 ± 0.25 mg.hr^2/ℓ v/s 0.83 ± 0.11 mg.hr^2/ℓ). Accordingly, a significant shorter mean residence time (MRT) than in normal state was observed in the dehydration. AUC, AUMC and MRT in both the states are given in Tables-XV, XVI and XVII respectively.

The fast disposition may be attributed to (i) accelerated renal function (more filtration or tubular secretion or blockage of back diffusion), (ii) enhanced metabolic activity or, (iii) reduced volume of distribution cleared by renal and metabolic routes at unaltered/enhanced clearance values. The first possibility may not be accepted because no significant change in the renal clearance of acetaminophen was observed during dehydration (Table-XI). Furthermore, total renal recovery of acetaminophen in the water deprived state was found to be significantly lower at $p \leq 0.05$ ($8.37 \pm 0.34\%$ v/s $6.97 \pm 0.55\%$). Total percent renal recovery of the administered dose in both the states is reported in Table-XVIII. The decrease in the recovery of the unchanged acetaminophen during water deprivation is consistent with the results of a previous study on rats.[93] It suggests that the reduction in renal excretion must be compensated most probably by metabolism. The second reason to explain fast disposition by metabolism seems to be more sound as a previous study[93] on rats has demonstrated higher glucuronidation of acetaminophen in water deprivation. As far as the

TABLE XV

TOTAL AREA UNDER THE PLASMA LEVEL VERSUS TIME CURVE OF ACETAMINOPHEN IN NORMAL AND WATER DEPRIVED SUBJECTS

Volunteer	AUC (ug.hr./ml/kg)	
	Normal	Water deprived
1	8.370	8.277
2	8.285	8.313
3	8.571	8.440
4	8.421	8.455
5	8.567	8.359
6	8.365	8.337
Mean ± SEM	8.43±8.85	8.36±8.83

ANALYSIS OF VARIANCE

S.O.V.	S.S.	D.F.	M.S.	F
Treatment (dehydration)	0.0132	1	0.0132	-
Residual	0.0926	10	0.0093	1.4194 ns
Total	0.1058	11	-	-

ns = No significant difference at p - 0.05

TABLE XVI

TOTAL AREA UNDER THE MOMENT CURVE (AUMC) OF ACETAMINOPHEN IN NORMAL AND WATER DEPRIVED SUBJECTS

Volunteer	AUMC ($\mu g.hr^2/ml/kg$)	
	Normal	Water deprived
1	1.581	0.674
2	0.922	0.698
3	2.481	1.005
4	0.946	1.310
5	1.960	0.761
6	1.232	0.533
Mean ± SEM	1.52±0.25	0.83±0.11

ANALYSIS OF VARIANCE

S.O.V.	S.S.	D.F.	M.S.	F
Treatment (dehydration)	1.4290	1	1.4290	-
Residual	2.2866	10	0.2287	6.2483+
Total	3.7156	11	-	-

+ = Significant difference at p - 0.05

TABLE XVII

MEAN RESIDENCE TIME OF ACETAMINOPHEN IN NORMAL AND WATER DEPRIVED SUBJECTS

Volunteer	Mean residence time (hr)	
	Normal	Water deprived
1	4.28	2.43
2	3.24	2.23
3	4.35	2.28
4	2.25	2.88
5	3.46	2.12
6	3.38	1.58
Mean ± SEM	3.49±0.31	2.25±0.17

ANALYSIS OF VARIANCE

S.O.V.	S.S.	D.F.	M.S.	F
Treatment (dehydration)	4.6128	1	4.6128	-
Residual	3.8731	10	0.38731	11.9101+
Total	8.4859	11	-	-

+ = Significant difference at p - 0.05

TABLE XVIII

RENAL RECOVERY OF ACETAMINOPHEN IN NORMAL AND WATER DEPRIVED SUBJECTS

Volunteer	Percent of dose recovered	
	Normal	Water deprived
1	7.52	9.58
2	8.38	6.75
3	8.71	7.06
4	9.53	6.19
5	7.33	6.45
6	8.74	5.78
Mean ± SEM	8.37±0.34	6.97±0.55

ANALYSIS OF VARIANCE

S.O.V.	S.S.	D.F.	M.S.	F
Treatment (dehydration)	5.88	1	5.88	-
Residual	12.57	10	1.26	4.68[ns]
Total	18.45	11	-	-

ns = Significant difference at p - 0.05

ANALYSIS OF VARIANCE**

S.O.V.	S.S.	D.F.	M.S.	F
Treatment (dehydration)	10.94	1	10.94	-
Residual	3.52	8	0.44	24.88[+]
Total	14.46	9		-

+ = Significant difference at p - 0.05

** Analysis without taking the values of volunteer 1 into the account.

contribution of reduced volume of distribution in fast disposition is concerned, it may not be possible to reject this completely. Total loss in volume of distribution does not seem to be more than 6%, as suggested by the body weight loss, whereas, the reduction in renal recovery which is more than 10% indicates accelerated metabolism.

3.2.4. Metabolic Excretion

The mean plasma levels of acetaminophen glucuronide (AG) and acetaminophen sulfate (AS) after administration of 600 mg oral dose of acetaminophen are given in Table-XIX. Time course of AG and AS in plasma (Figures 5 and 6) reflects gradual increase and then decline of plasma concentrations of the metabolites. No glucuronide metabolite could be detected in the plasma of any of the volunteers at 15 minutes after drug administration and a very significant decrease/non-existence was noted at $p \leq 0.002$. AG starts appearing in the plasma half an hour after the administration of the drug, the levels being significantly lower ($p \leq 0.02$) than those of the normal ones. The lower levels in the initial phase of profile are also similarly observed in AS pattern during dehydration. Contrary to this resemblance, an opposite effect of higher concentration in the last portion of AG profile was noted. The plasma levels of AG at fourth and sixth hours in the water deprived subjects were significantly higher ($p \leq 0.05$) than those observed in the normal subjects. Similar to AG no sulfate could be detected 15

PLASMA LEVELS OF ACETAMINOPHEN GLUCURONIDE AND ACETAMINOPHEN SULFATE IN NORMAL AND WATER DEPRIVED HUMANS (n=6) AFTER ORAL ADMINISTRATION OF SINGLE DOSE OF 600 mg ACETAMINOPHEN

Time (hours)	Plasma levels (mean + SEM), ug/ml					
	Acetaminophen glucuronide		Significant* difference	Acetaminophen sulfate		Significant* difference
	Normal	Water deprived		Normal	Water deprived	
0.25	1.16 ± 0.20	0.00 ± 0.00	$p \leq 0.002$	1.90 ± 0.24	0.00 ± 0.00	p 0.001
0.5	2.27 ± 0.15	0.68± 0.43	$p \leq 0.02$	2.86 ± 0.33	1.35 ± 0.37	p 0.05
1	5.84 ± 0.95	4.04± 0.99	ns	3.38 ± 0.37	2.55 ± 0.47	ns
2	8.60 ± 1.01	8.06± 0.92	ns	3.17 ± 0.33	2.87 ± 0.34	ns
4	4.86 ± 0.34	7.58±0.64	$p \leq 0.001$	1.90 ± 0.25	2.19 ± 0.28	ns
6	3.59 ± 0.19	4.38±0.49	$p \leq 0.05$	1.20 ± 0.25	0.67 ± 0.22	p 0.05

* Evaluated through 7-test.

ns No significant difference.

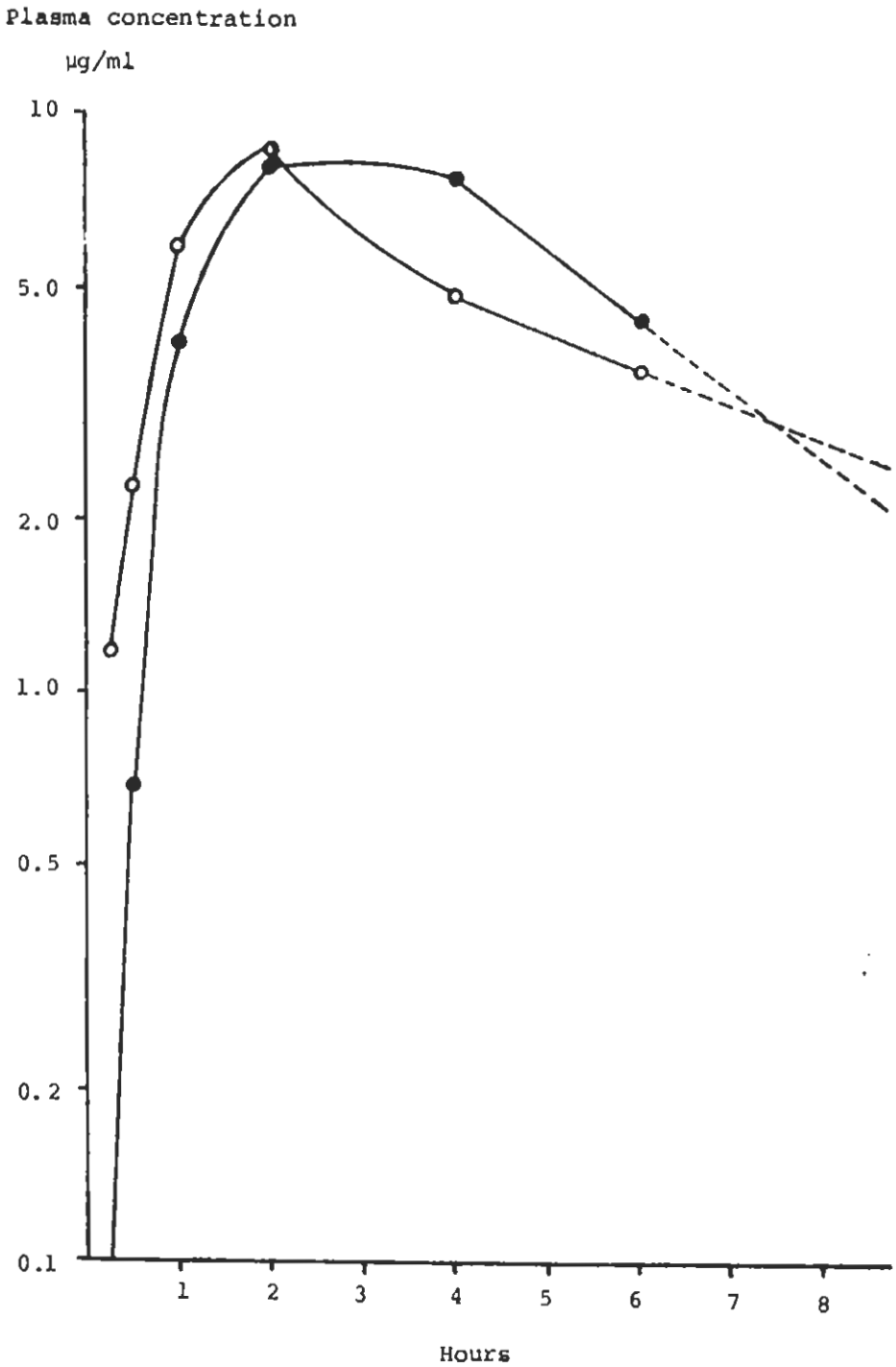


Figure 5 - Mean plasma level versus time profiles of acetaminophen glucuronide in normal and water deprived (24 hours) states of humans (n=6). Key: o— Normal ●— Water deprived.

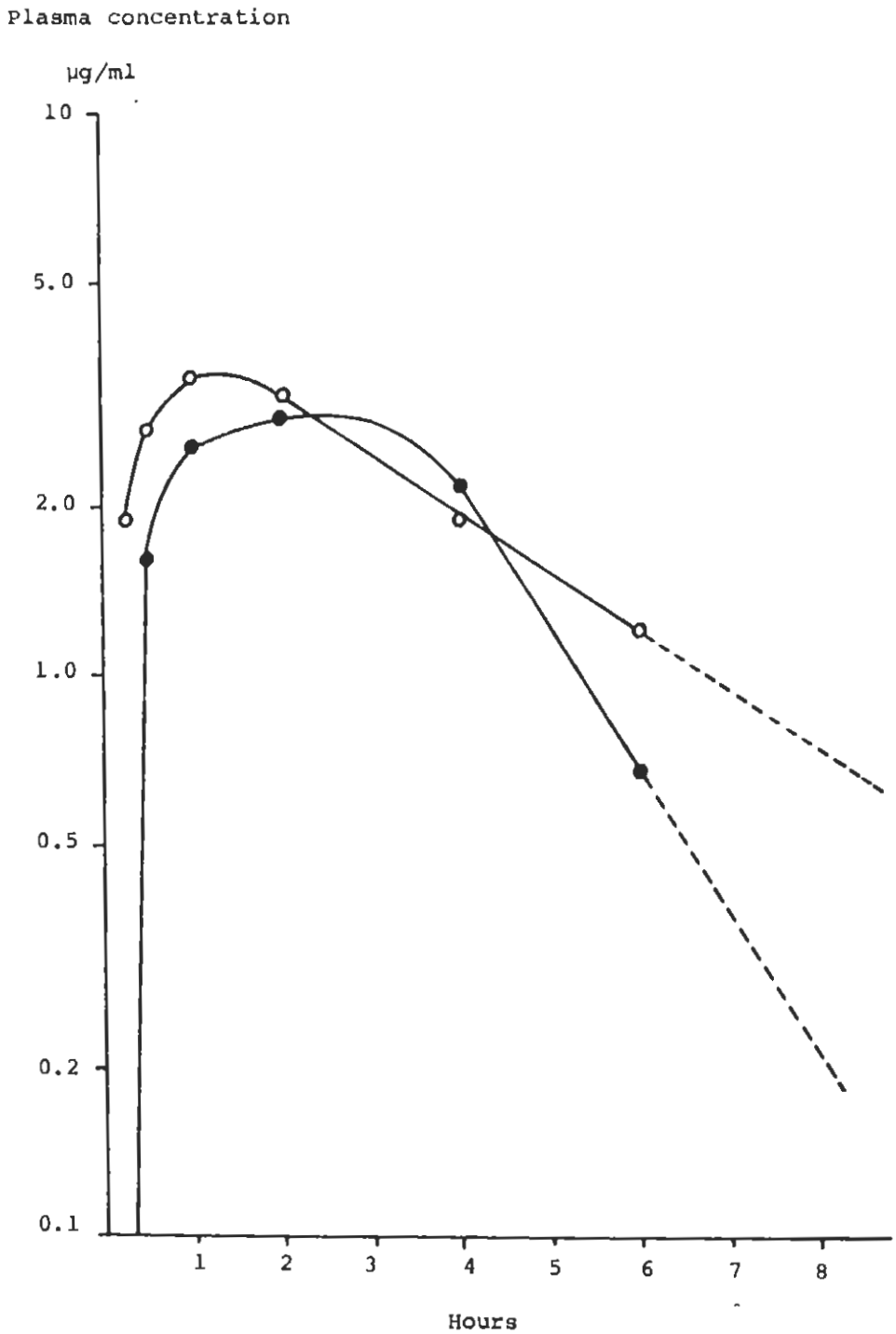


Figure 6 - Mean plasma level versus time profiles of acetaminophen sulfate in normal and water deprived (24 hours) states of humans (n=6). Key: o— Normal ●—Water deprived.

minutes after the drug administration in the first plasma sample of any of the volunteers during dehydration. The probability of non-existence was 0.001. After half an hour small concentrations of AS appeared in plasma of the water deprived subjects which were significantly lower (2.86 mg/ℓ v/s 1.35 mg/ℓ) at $p \leq 0.05$. The plasma levels of AS one hour after administration of the drug remained insignificantly different from those of the normal levels. At the sixth hour significantly lower levels in the water deprived subjects were again noted with AS at $p \leq 0.05$.

From the above discussion regarding the pattern differences the following inferences may be drawn. During dehydration the absorption of acetaminophen in its very initial stage (up ≈ 20 minute) slows down in comparison to that of the normal state and, therefore, little formation of metabolites could be reported. This results in non-detectable levels of AG and AS. However, with the course of time there remains no appreciable difference in plasma levels of the normal and water deprived subjects. This situation remains upto four hours after the drug administration. From this stage onwards a significant increase in AG levels in comparison to that of the normal levels reflects either higher glucuronidation or some retardation in renal clearance of AG in dehydration. On the other hand, a significant decrease in AS levels in the last portion of concentration versus time profile may be explained either by net effect of higher

glucuronidation or by slow rate of sulfation or by accelerated renal clearance of AS. The areas under the curve upto six hours, AUC_{o-6}, of AG and AS are reported in Tables XX and XXI. No significant difference at $p \leq 0.05$ in the AUC_{o-6} of AG and AS was found during water deprivation.

The renal recoveries of AG, AS in terms of percent dose recovered of the total renal recovery are given in Tables XXII and XXIII. The metabolic process of glucoronidation is two fold faster than that of the process of sulfation. In the normal subjects AG contributed 59.52 $\pm$ 3.76% of the total renal recovery, whereas AS contributed 25.59 $\pm$ 2.55%. These results are in good agreement with the findings of previous studies.[108,111,112,129,133,134] The increase in total percent recovery of AG and AS statistically is not significant.

Glucuronide and sulfate conjugates of acetaminophen are completely excreted through renal route. Hence their renal clearance is equivalent to their total body clearance. The renal clearance data (Tables XXIV and XXV) indicates that appreciably high changes occur in renal clearance of AS. Renal clearance of AS was found to be more than double to the normal state.

The mechanism for renal clearance cannot be fully understood until the creatinine clearance, urine flow-rate and plasma concentration are not taken into the account. The regression

TABLE XX

AREA UNDER THE PLASMA LEVEL CURVE OF ACETAMINOPHEN GLUCURONIDE IN NORMAL AND WATER DEPRIVED SUBJECTS

Volunteer	AUC_{0-6} (µg.hr/ml/kg)	
	Normal	Water deprived
1	0.359	0.355
2	0.421	0.522
3	0.429	0.594
4	0.618	0.705
5	0.488	0.419
6	0.529	0.715
Mean ± SEM	0.47±0.04	0.55±0.06

ANALYSIS OF VARIANCE

S.O.V.	S.S.	D.F.	M.S.	F
Treatment (dehydration)	0.0181	1	0.0181	-
Residual	0.1512	10	0.0151	1.1984^{ns}
Total	0.1693	11	-	-

ns =No significant difference at p - 0.05

TABLE XXI

AREA UNDER THE PLASMA LEVEL CURVE OF ACETAMINOPHEN SULFATE IN NORMAL AND WATER DEPRIVED SUBJECTS

Volunteer	AUC_{0-6} (µg.hr/ml/kg)	
	Normal	Water deprived
1	0.260	0.048
2	0.206	0.064
3	0.196	0.063
4	0.262	0.246
5	0.274	0.180
6	0.110	0.230
Mean ± SEM	0.22±0.03	0.14±0.04

ANALYSIS OF VARIANCE

S.O.V.	S.S.	D.F.	M.S.	F
Treatment (dehydration)	0.0190	1	0.0190	-
Residual	0.0602	10	0.0060	3.1601 ns
Total	0.0792	11	-	-

ns =No significant difference at p - 0.05

TABLE XXII

RENAL RECOVERY OF ACETAMINOPHEN GLUCURONIDE IN NORMAL AND WATER DEPRIVED SUBJECTS

Volunteer	Percent of dose recovered	
	Normal	Water deprived
1	62.46	47.02
2	43.38	42.86
3	19.35	65.61
4	23.83	27.95
5	34.76	34.34
6	41.48	39.85
Mean ± SEM	37.54±6.32	42.94±5.29

ANALYSIS OF VARIANCE

S.O.V.	S.S.	D.F.	M.S.	F
Treatment (dehydration)	87.3	1	87.3	-
Residual	2035.9	10	203.6	0.4289^{ns}
Total	2123.2	11	-	-

ns=No significant difference at p - 0.05

TABLE XXIII

RENAL RECOVERY OF ACETAMINOPHEN SULFATE IN NORMAL AND WATER DEPRIVED SUBJECTS

Volunteer	Percent of dose recovered	
	Normal	Water deprived
1	16.22	16.68
2	11.16	12.51
3	8.99	21.24
4	14.87	13.74
5	19.50	19.02
6	21.76	20.58
Mean ± SEM	15.42±1.98	17.30±1.47

ANALYSIS OF VARIANCE

S.O.V.	S.S.	D.F.	M.S.	F
Treatment (dehydration)	10.58	1	10.58	-
Residual	182.52	10	18.25	0.5799^{ns}
Total	193.10	11	-	-

ns=No significant difference at p - 0.05

TABLE XXIV

RENAL CLEARANCE OF ACETAMINOPHEN GLUCURONIDE IN NORMAL AND WATER DEPRIVED SUBJECTS

Volunteer	Renal clearance (ml/hr/kg)	
	Normal	Water deprived
1	249.10	195.4
2	129.90	107.3
3	69.26	181.2
4	58.29	65.0
5	96.65	117.4
6	105.60	81.7
Mean ± SEM	118.13+28.21	124.67+21.57

ANALYSIS OF VARIANCE

S.O.V.	S.S.	D.F.	M.S.	F
Treatment (dehydration)	128.1	1	128.1	-
Residual	37838	10	3783.8	0.034^{ns}
Total	37966	11	-	-

ns= No significant difference at p - 0.05

TABLE XXV

RENAL CLEARANCE OF ACETAMINOPHEN SULFATE IN NORMAL AND WATER DEPRIVED SUBJECTS

Volunteer	Renal clearance (ml/hr/kg)	
	Normal	Water deprived
1	86.13	516.39
2	68.15	94.23
3	70.29	181.16
4	85.67	191.07
5	96.62	342.39
6	265.77	247.50
Mean ± SEM	112.11±31.04	262.12±60.85

ANALYSIS OF VARIANCE

S.O.V.	S.S.	D.F.	M.S.	F
Treatment (dehydration)	67517	1	67517	-
Residual	140007	10	14001	4.8224^{ns}
Total	207524	11	-	-

ns = Significant difference at p - 0.05

analyses of diuresis versus renal clearance and plasma concentration (taken as average area under the curve, $AUC_{o-6}/6$) versus renal clearance data were performed. The values of slopes (b), regression coefficients (r) and significance of correlation are reported in Table XXVI. As a rule, estimate of a significant correlation and a positive value of slope indicate the dependence of renal clearance upon the glomerular filtration rate and effective reabsorption. In fact, in the present investigation the water deprived subjects excreted acetaminophen slower than the normal ones, which is mainly caused by the extreme low urine flow (0.29 ml/min in dehydration v/s 0.97 ml/min in normal state). Thus it appears that tubular reabsorption is the governing mechanism as supported by the significant correlation between diuresis and clearance of APAP and by a positive slope (Table XXVI).

In order to restore a high renal clearance so as to ensure a maximal elimination rate, the acetaminophen becomes conjugated. As soon as the reabsorption by the free OH group is blocked by the presence of a conjugated group, the compound is again excreted by active tubular secretion. Renal clearance of creatinine in water deprived subjects was found to be 81.21 ml/hr/kg; which is much smaller than the renal clearance of AG and AS in water deprivation and bigger than of APAP. This proves an active tubular secretion mechanism for AG and AS and its non-effectiveness for APAP. This is in agreement with earlier studies

CORRELATION COEFFICIENT AND SOLPE VALUES OBTAINED THROUGH REFRESSION ANALYSIS OF RENAL CLEARANCE WITH DIURESIS AND PLASMA CONCENTRATION.

Product	Slope	Correlation	Signficance of correlated coefficient.	Comments
RENAL CLEARANCE: DIURESIS				
Acetaminophen glucuronide	-0.0098	0.258	ns	Filteration and reabsorption have insignificant role in renal clearance
Acetaminophen sulfate	-0.028	0.366	ns	
Acetaminophen	0.0047	0.573	$p \leq 0.1$	Renal clearance is urine-flow dependent. Reasorption is indicated.
RENAL CLEARANCE: PLASMA CONCENTRATION				
Acetaminophen glucuronide	-0.751	0.340	ns	-
Acetaminophen sulfate	-6.00	0.738	$p \leq 0.05$	High active secretion.
Acetaminophen	-0.536	0.689	$p \leq 0.05$	Active secretion.

ns = No significant correlation at $p \leq 0.05$

which determined glomerular filtration and reabsorption of APAP,[117] tubular secretion for AS[118,123] and both tubular secretions and reabsorptions for AG.[117]

Regression analysis of plasma concentration of AS versus its renal clearance data gives a significant correlation co-efficient ($p \leq 0.05$) with a high negative slope (-6.0) indicating a very high active tubular secretion (Figure 7). The serum protein binding of APAP, AG and AS are ≈ 20%, >20% and >50% respectively.[118] Thus a correction of renal clearances of APAP and AG for protein binding would not be appreciably significant. Whereas, by considering more than 50% serum protein binding of AS the renal clearance referenced to the unbound metabolite would be more than double. This confirms the definite renal tubular secretions of AS. Similar regression analysis for AG shows a significant correlation ($p \leq 0.05$) coefficient with a negative slope value of 0.538. It suggests that although active tubular secretion is the main effective excretory process for renal clearance of AG but its mechanism is different and the rate is slow as compared to that of AS. During dehydration an increase in renal clearance of AS is more than two fold (112.11 ml/hr/kg v/s 262.12 ml/hr/kg) whereas no change is observed in renal clearance of AG. This further supports that the active tubular secretion mechanism for AS is different from that of AG. This conclusion is also favored, because probencid has been found to inhibit the net renal

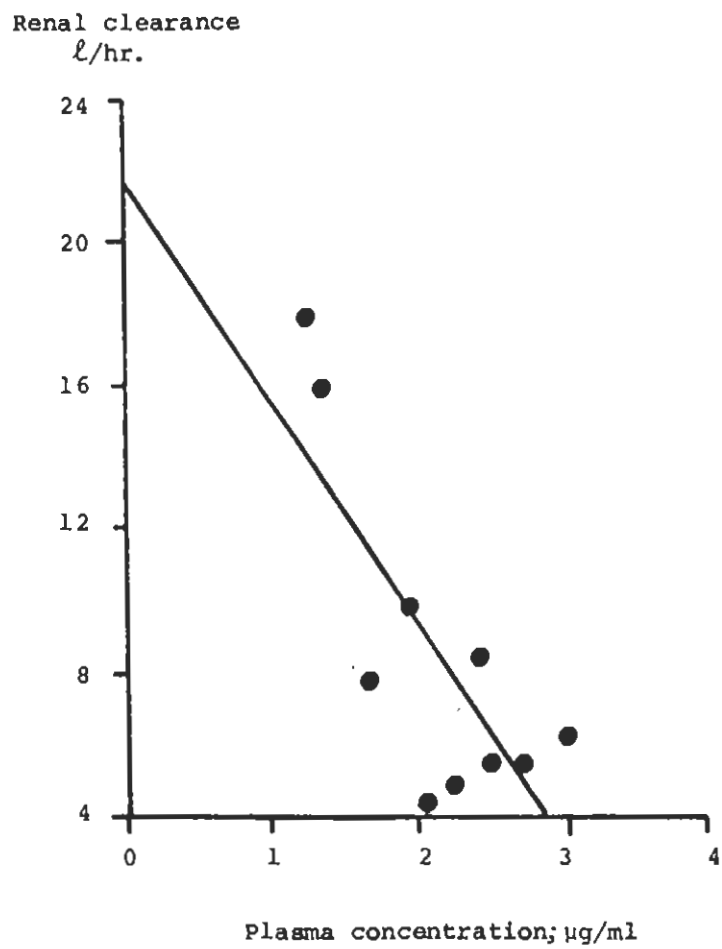


Figure 7 - Renal clearance of acetaminophen sulfate and its plasma concentration.

tubular secretion of acetaminophen sulfate but not that of the glucuronide in dogs.[117]

The possible different mechanism seems due to the different binding sites of enzyme engaged in tubular secretion. There is no question of increased filtration, due to slow GFR in water deprivation as revealed by creatinine clearance and due to low urine flow rate. Slower reabsorption or blockage of reabsorption are also nonindicative as low plasma volume (most probably) and lower urine flow rate provide more chances for reabsorption. There remains only one possibility of higher tubular secretory mechanism of AS than with AG.

4. SUMMARY AND CONCLUSION

The main features of the present investigation may be summarized as follows:

In humans the effect of water deprivation upto 24 hours on thirty eight physiologic and biochemical characteristics has been evaluated. The effect of dehydration on pharmacokinetics of acetaminophen and the excretion of its two major metabolites i.e. acetaminophen glucuronide and acetaminophen sulfate has also been studied. The physiologic and biochemical parameters included the study of weight, hematology, serum proteins, acid-base status, blood gas content, renal and hepatic functions, enzyme levels and electrolyte levels as well as their clearances.

From an overall view of the changes occurring in the physiologic and biochemical system, it appears that the very first parameter being affected is the serum osmolality which reached upto 320 mosmol/kg from 291 mosmol/kg after 24 hours. Besides severe thirst as a consequence of high osmolality, the body remained under high stress showing lack of mobility of volunteers and a significant* decrease in the levels of CK and GOT. Similarly a significant increase in neutrophil count and significant decrease in lymphocyte count also indicate the stress over the body.

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*The word "significant" in this text is used to indicate significant change at $p \leq 0.05$.

The second important change comes in the renal physiology. The creatinine clearance, an index of glumerular filteration rate, is affected severely and reduced upto 5.16 ± 0.14 1/hr from 7.68 ± 0.34 1/hr. Similarly, the urine flow rate also decreases upto the minimum possible limit of 17.16 ± 1.38 ml/hr with 1400 mosmol/kg urine osmolality which in turn is also the maximum possible urine osmolality. These significant changes in renal physiology during dehydration are of great value for determining the dosage regimen of drugs whose disposition mainly depends upon the tubular filteration. As in the water deprivation state the input and output balance of water is disturbed, the hemoconcentration and a significant reduction in the body weight (3.17 = 0.28%) occur. Hemoconcentration is quite evident from the significant increase in the hematocrit value (41.8 ± 1.39 vs 47.0 ± 2.21) and sodium ion concentration (141.6 ± 1.46 vs 155.6 ± 2.14 mosmol/1). However, the increased sodium ion concentration also depicts the efforts of the body to retain the maximum possible water.

With a reducing quantity of water in different fluid compartments, a significant increase in the renal clearance of electrolytes (sodium and potassium ions) takes place in order to maintain the physiological osmolality, whereas the increase in sodium ion renal clearance (17.06 ± 5.17 vs 77.62 ± 11.7 ml/hr) and potassium on renal clearance (0.47 ± 0.09 vs 0.66 ± 0.06 ml/hr) indicates the water loss from extracellular and

intracellular fluid compartments respectively. And hence the water loss occurs in both the compartments and none of the compartment is saved preferentially. Due to dehydration no significant change was observed in the total proteins, albumin and α_1 and γ globulins. However, α_2 and β globulins were found to be significantly increased which may be explained either in terms of the stress or destruction of red blood cells which might takes place due to hyperosmolality. Alternatively the increase may be explained by disfunctioning of either liver or kidney or of both. However, the liver malfunctioning could not be detected by any of the parameters evaluated. Furthermore, no change of any clinical significance occurred in the blood levels of glucose, urea, triglycerides and total lipids.

Although the blood pH, due to very effective blood buffering system, remained unaffected, no change in the oxygen and carbon dioxide pressures was noted, the actual bicarbonate, base excess and total carbon dioxide were found to be significantly increased (11.11%, 32.38% and 11.23% respectively). This reveals a tendency towards metabolic alkalemia which may be more profound after prolonged dehydration and may be responsible for significant changes in the pharmacokinetics of drugs.

No significant change was observed in the absorption half-life (12.53 $\pm$ 1.75 vs 15.62 $\pm$ 2.75 minutes) of the drug in water deprivation state, however, the significanty lower blood

levels (5.25 ± 0.79 vs 2.66 ± 0.97µg/ml) were found at the first sampling time of 0.25 hours. No change occurred in the peak plasma level and in the time of the maximum drug concentration. The disposition of acetaminophen was faster in water deprivation than that of the normal state. The mean residence time in dehydration was found to be significantly short (3.4 ± 0.31 vs 2.25 ± 0.17 hr). Although the renal clearance in both the states was insignificantly different, the percent of the intact drug recovered in urine during dehydration was appreciably small (8.37 ± 0.34 vs 6.77 ± 0.55%). This indicates that the enhanced metabolism of the drug in the water deprivation state is responsible for the faster disposition of the drug.

Under water deprivation the plasma levels of acetaminophen glucuronide (AG) and acetaminophen sulfate (AS) were significantly lowered in the initial phase (at 0.25 and 0.5 hr.). Areas under the curve and percent amount of the dose recovered in urine in the form of AG and AS were insignificantly different in the normal and water deprived states. However, the renal clearance of AS was appreciably high as compared with that of the normal state (112.11 ± 31.04 vs 262.12 ± 60.85 ml/hr/kg).

The comparison of renal clearance values of acetaminophen and its metabolites with that of creatinine clearance reveals that tubular reabsorption is the main effective process for acetaminophen renal clearance, whereas tubular secretion is the main excretory mechanism of the metabolites. The correlation

between the renal clearance and diuresis indicates that renal clearance of acetaminophen is urine-flow dependent whereas the filteration and reabsorption are least important in the renal excretion of AG and AS. The regression analysis of renal clearance and plasma levels confirmed active secretion of AG, AS and acetaminophen. However, the process is more profound for AS than AG. This suggests that the active tubular secretion mechanism for AS is different from that of AG. This is further supported by the effect of dehydration due to which the renal clearance of AS was increased more than twice whereas that of AG remained almost constant.

APPENDIX I

COMPUTING PROGRAM FOR NONCOMPARTMENTAL ANALYSIS

To perform the noncompartmental pharmacokinetic analysis of the blood levels, a programable calculator, Casio FX-602 P, was employed. For this analysis a program was written which is capable to process the plasma levels obtained after intravenous administration and the levels obtained after first order input as well. The following equations were employed to calculate different parameters:

Area under the curve; $AUC_{0-t} = 0.5\,|(t_2-t_1)(c_1+c_2)| + 0.5|(t_3-t_2)(c_2+c_3)| \ldots + 0.5|(t_n-t_{n-1})(c_{n-1}+c_n)|$

Area under the first moment curve; $AUMC_{0-t} = 0.5\,|(t_2-t_1)(c_1t_1+c_2t_2)| + 0.5\,|(t_3-t_2)(c_2t_2+c_3t_3)| \ldots + 0.5\,|(t_n-t_{n-1})(c_{n-1}t_{n-1}+c_nt_n)|$

Total area under the curve; $AUC_{0-\alpha} = AUC_{0-t} + c_t/\beta$

Total area under the first moment curve; $AUMC_{0-\alpha} = AUMC_{0-t} + c_t.t + c_t/\beta$

Mean residence time; $MRT = AUMC_{0-\alpha} / AUC_{0-\alpha}$

Elimination rate constant; β (Slope of the terminal phase) $= \Sigma x\ell ny - 1/n\Sigma x\ell ny / \Sigma x^2 - 1/n\,(\Sigma x)^2$

Correlation coefficient; $r = a\,\Sigma\ell ny + b\Sigma x\ell ny - 1/n\,(\Sigma\ell ny)^2 / \Sigma\ell ny - 1/n\,(\Sigma\ell ny)^2$

Where;

c = Plasma concentration
t = Time of sampling
n = Number of sampling
a = Intercept of terminal phase on y axis

The print-out of the program list alongwith the print-out of the plasma levels, data of volunteer #1 and kinetic estimates are given in Table XXVII. Flow chart shown in Figure 8 illustrates the sequence of computing operation.

TABLE XXVII

PROGRAM LIST FOR THE NONCOMPARTMENTAL PHARMACOKINETIC COMPUTATION OF PLASMA LEVELS ON PROGRAMABLE CALCULATOR CASIO FX -602 P

```
PROGRAM LIST
M00-23.F-1F 480steps

*** P0
"Time     Conc"
SAVE invEXE
MAC ) Min00
LBL1
MR00
HLT Min03 ≠ 0
HLT Min04 = Min21
GSBP1
GOTO1
LBL2
MR00
HLT Min03 xo ≠ 0
HLT Min04 in M+02
MinF = M+01 M+11
MRF x² M+16
MR03 ÷ MR04 = Min21
GSBP1
GOTO2
LBL3
GSBP2
MR19 FIX2
"AUC="
SAVE invEXE
MR22 FIX2
"AUMC="
SAVE invEXE
MR15 - MR01 = Min15
M+19
MR19 FIX2
"AUC(total)="
SAVE invEXE
MR15 x MR18 = + MR15
÷ MR01 = M+22
MR22 FIX2
"AUMC(total)="
SAVE invEXE
MR22 ÷ MR19 = FIX2
"MRT="
SAVE invEXE
        ···161steps
```

```
PROGRAM LIST
M00-23.F-1F 480steps

*** P1
" MR03      MR04 "
SAVE invEXE
TSZ .5 x ( MR03 -
MR18 ) = Min23 ≠ (
MR15 + MR04 ) = M+19
( MR20 + MR21 ) x
MR23 = M+22
MR04 Min15
MR21 Min20
MR03 Min18
        ···053steps

PROGRAM LIST
M00-23.F-1F 480steps

*** P2
" "
SAVE invEXE
"RESULTS"
SAVE invEXE
MR02 Min03 x² Min12
( MR01 - x̄ x MR02 )
÷ ( MR07 - x̄ x MR08
) = Min01
( MR02 - MR01 x MR08
) ÷ MR09 = Min02 x
MR03 + MR01 x MR11 -
MR12 ÷ MR09 = ÷ (
MR16 - MR12 ÷ MR09 )
=
"r="
SAVE invEXE
"K= MR01 "
SAVE invEXE
MR01 +/- Min01
MR02 eˣ
"a="
SAVE invEXE
        ···095steps
```

Workd example

```
Time     Conc
0.25     5.97
0.5     14.82
1       20.94
2       19.63
4       12.53
6        4.81

RESULTS
r=0.958242535
K=-0.35159048
a=43.1632483
AUC=82.87
AUMC=206.85
AUC(total)=95.75
AUMC(total)=327.84
MRT=3.42
```

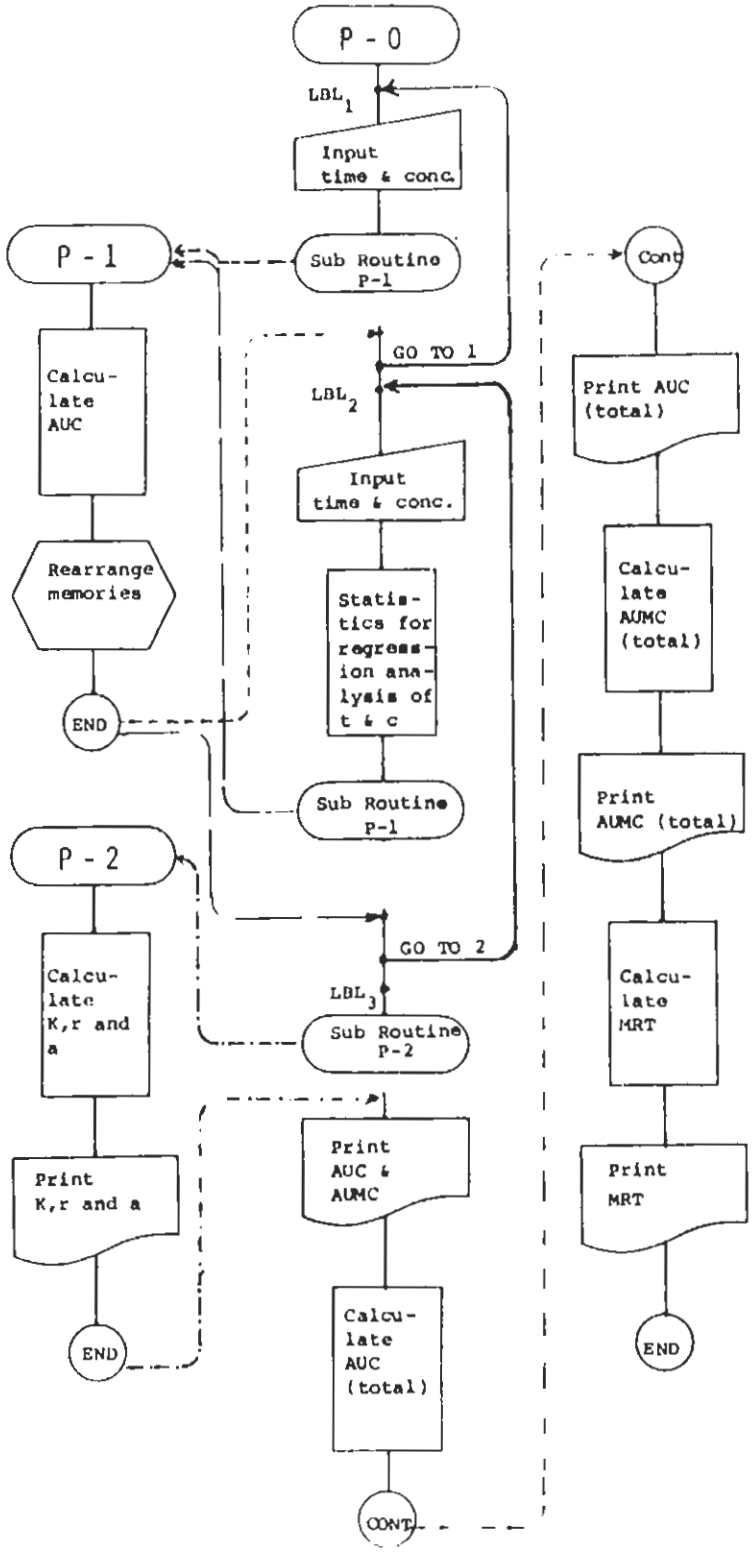

Figure 8 - Flow chart of computing program for noncompartmental pharmacokinetic analysis.

APPENDIX II

COMPUTING PROGRAM FOR STATISTICAL ANALYSIS

To determine the significance of the difference, among different pharmacokinetic, physiologic and biochemical parameters, data was processed by a programmable calculator Casio FX-602 P. A computing program for this purpose was written. The program is capable to calculate the mean, SEM and variance ratio of control and treatment sets of data before performing the analysis of variance (ANOVA). After doing ANOVA, the program applies both the paired and unpaired tests on the data to estimate 't' values.

The print-out of the program list alongwith a print-out of worked example, which contains data and the estimates of the above mentioned statistics, are given in the Table XXVIII. The following equations were employed to estimate the statistics.

Mean; $\bar{x}$ or $\bar{y} = \Sigma x/n$ or $\Sigma y/n$

Standard error of mean; SEM = $\delta_{n-1}/\sqrt{n}$

Variance ratio; $F = \delta^2_{n-1}/\delta^2_{n-1}$

ANALYSIS OF VARIANCE

Sum of squares, SS;

$$SS_{treat.} = n_x|\bar{x}-(\bar{x}+\bar{y})/2|^2 + n_y\ |y-(\bar{x}+\bar{y})/2|^2$$

$$SS_{error} = \Sigma\ (x-\bar{x})^2 + \Sigma\ (y-\bar{y})^2$$

$$SS_{total} = SS_{treat.} + SS_{error}$$

Degree of freedom, DF;

$$DF_{treat.} = 2-1 = 1$$

$$DF_{error} = (n_x - 1) + (n_y - 1)$$

Mean square, MS = SS/DF

Variance ratio; $F = MS_{treat.}/MS_{error}$

t-TESTS

t-test (paired); $t = \bar{d}/\delta_{dn-1}\sqrt{n}$

Where;

d = Difference between paired values

$\bar{d}$ = Average difference

n = Number of pairs

t-test (unpaired); $t = \bar{x} - \bar{y}/s \cdot n_x n_y / n_x + n_y$

Where;

$$s = |\Sigma(\bar{x}-x_i)^2 + \Sigma(\bar{y}-y_i)|^2/n_x + n_y - 2$$

The flow chart shown in Figure 9 illustrates the sequence of computing operations.

TABLE XXVIII

PROGRAM LIST FOR F-TEST, ANALYSIS OF VARIANCE (ANOVA) AND t-TESTS ON PROGRAMABLE CALCULATOR CASIO FX-602 P

```
TASNEEM  27.11.1984

PROGRAM LIST
M00-23,F-1F 498steps

*** P0
SAVE invEXE
MAC
"Normal      Deprive
d"
SAVE invEXE
LBL1
MR09 + 1 =
HLT Min01 xD - 0
HLT Min02 M+11 x²
M+12 ∫ = M+21 x²
M+22
" AR01          AR02
"
SAVE invEXE
GOT01
        ...065steps

*** P1
MR09 - 1 = Min01
x̄ Min03
σn-1 x² Min02
SAC MR01 + 1 = Min09
MR11 Min08
MR12 Min07
MR02 ∫ ÷ MR09 ∫ =
FIX2 MinF
σn-1 ÷ MR09 ∫ = FIX2
Min1F
MR03 FIX2
"Mean+/-SEM"
SAVE invEXE
"Nor. #+/- ARF "
SAVE invEXE
x̄ FIX2
"Deh. #+/- AR1F "
SAVE invEXE
GSBP2
        ...087steps

*** P2
"  ----------"
SAVE invEXE
MR02 ÷ σn-1 x² = FIX4
"F-ratio      #"
SAVE invEXE
"     A N O V A"
SAVE invEXE
x̄ + MR03 = ÷ 2 =
Min05 - x̄ = x² x
MR09 = M+06
MR05 - MR03 = x² x
MR09 = M+06
MR06 FIX4
"SS(treatment)#"
SAVE invEXE
"(s)2 Treat   #"
SAVE invEXE
MR02 x MR01 + σn-1 x²
x MR01 = Min14 FIX4
"SS(error)    #"
SAVE invEXE
MR14 ÷ 2 ÷ MR01 =
Min15 FIX4
"(s)2 error   #"
SAVE invEXE
1/x x MR06 = FIX4
"F =          #"
SAVE invEXE
"      t-Tests"
SAVE invEXE
( MR02 x MR01 ) + (
σn-1 x² x MR01 ) = ÷
( MR01 x 2 ) = ∫
Min04
MR01 x 2 + 2 = Min15
1/x x MR09 x² = ∫ x
( MR03 - x̄ ) ÷ MR04
= FIX4
"t (unpaired) #"
SAVE invEXE
SAC MR01 + 1 = Min09
MR21 Min08
MR22 Min07
x̄ ÷ σn-1 x MR09 ∫ =
FIX4
"t (paired)   #"
SAVE invEXE
        ...328steps
```

Workd example

```
AAAA-Peak concentrat
ion
Normal      Deprived
5.21        5.24
6.53        5.82
10.9        8.43
7.68        8.19
9.84        10.5
8.56        9.94
Mean+/-SEM
Nor. 7.99+/-0.81
Deh. 8.14+/-0.8
  ----------
F-ratio      1.0787
    A N O V A
SS(treatment)0.0675
(s)2 Treat   0.0675
SS(error)   38.9701
(s)2 error   3.8970
F =          0.0173
     t-Tests
t (unpaired) -0.1316
t (paired)   -0.2444
```

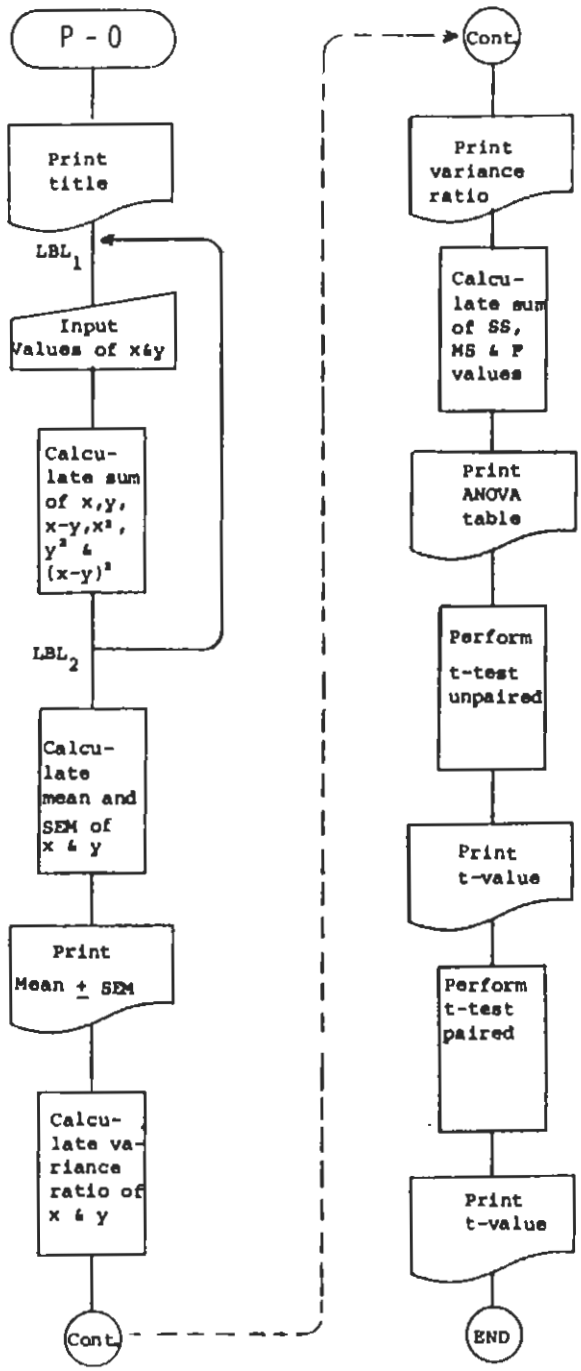

Figure 9 - Flow chart of computing program for F-test, analysis of variance (ANOVA) and t-tests.

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