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SPECIAL WEAPONS PROJECT
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TITLE OF PROJECT: Intracellular Changes in Trauma, Depletion and Repair - with Special Reference to Burns

Objectives: The improvement of the therapy of trauma through study of its cellular and metabolic effects. Since 1950 the investigations have centered on the problem of burns. Patients with endocrine diseases such as Cushing's disease and with perforated duodenal ulcers have been included. The studies have been carried out both at fundamental physiologic and at practical clinical levels.

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ABSTRACT (OR SUMMARY) OF RESULTS

I. Since Start of Project

A. 1948-1949 - The first $2\frac{1}{2}$ years of the project were spent largely in the investigation of methods and the setting up of laboratory routine. In the second half of this period with the departure of Dr. Francis D. Moore to the Peter Bent Brigham Hospital the budget and technical personnel were divided.

A first step was the establishment of a metabolism ward for the study of patients with surgical diseases. The scope and facilities of the chemistry laboratory were enlarged. The flame photometer was introduced for the measurement of potassium and sodium. Much time was spent in trying to adapt this instrument to the measurement of magnesium and calcium, but without avail because of the low biological concentrations.

A full fledged isotope laboratory was organized. Professional and technical personnel were trained under the guidance of consulting physicists. Adequate instrumentation has gradually been achieved.

Four fields of isotope investigation were initiated and developed during these first years. The technic of measuring the radioactive sodium space was mastered. Radioactive phosphorus was used in the measurement of renal function and the metabolic activity of tissues. Dr. F. D. Moore introduced radioactive potassium and deuterium oxide to measure the intracellular space and the total body water.

The surgical diseases studied were various. Dr. F. D. Moore studied the effect of operation and a number of surgical conditions, including those associated with malnutrition. These studies formed the basis of his monograph, Moore F. D. and M. R. Ball: The Metabolic Response to Surgery, Springfield, Illinois, Charles C Thomas, 1952, 156 pp.

At the Massachusetts General Hospital metabolic aspects of Cushing's disease were studied. Principal attention was given the measurement of the sodium space before and after successful surgical relief by adrenalectomy. No abnormality of the sodium space was determined in the patients studied.

The equilibration rate and renal excretion of P-32 were measured in patients with hyperparathyroidism in the hope of finding a more sensitive test for the diagnosis of this disease. These thoughts proved illusory. An increased uptake of P-32 was found in the parathyroid adenomas as compared with normal parathyroid tissue. A comparable increase in P-32 uptake was found in other endocrine tumors including those of the thymus, adrenal and thyroid.

The study of the metabolic effect of a perforated peptic ulcer was initiated during this period. (See II. Current Report Period, and Appendix F.)

B. 1950-1952 - With the rearmament program and the outbreak of the Korean War the work of this project was enlarged and reoriented to the problem of burns. Dr. Bradford Cannon, plastic surgeon to this hospital, and others joined the project.

The therapeutic possibilities of ACTH and cortisone were an immediate concern. Their use in the shock therapy and in wound healing were investigated in experimental animals and burned patients. The Drinker lymph preparation in the foot of the dog and the rate of development of blebs in the skin of young hogs were used as experimental preparations to study the effect of these hormones on the abnormal capillary permeability engendered by a burn. The hormones were found not to influence the rate of filtration or the protein concentration of the fluid seeping through the damaged

capillaries. (Wight, A., P. A. Weisman, R. L. Rovit and O. Cope: Adrenal Hormones and Increased Capillary Permeability of Burns. A.M.A. Arch. Surg. 65: 309, 1952)

The rates of edema formation and edema resorption were measured in a sterile plethysmograph as the best method of approaching this problem in the human being. The burned hands of patients treated with ACTH or cortisone developed as much edema as the control burned hands of patients without hormone therapy. The length of time taken to reach the peak of edema was as long in the treated as in the untreated patients. The rate of subsidence of the edema was comparable in the two groups of patients. Had the adrenal hormones been effective in limiting the seepage from the capillaries, and thus in diminishing the amount of fluid needed as therapy, the rate of edema formation would have been less. Had the hormones brought about early healing of the damaged capillaries the time at which the peak of edema was reached would have been advanced and presumably the subsidence of edema hastened. Experimental burns were made on patients with Cushing's disease and normal volunteers. The same rate of bleb formation and healing was observed in the two groups. The only possible conclusion from all these observations was that ACTH and cortisone were without demonstrable effect or therapeutic usefulness in this aspect of burn therapy. (Raker, J.W., A. Wight, A.J.D. Michel and O. Cope: A Clinical and Experimental Evaluation of the Influence of ACTH on the Need for Fluid Therapy of the Burned Patient. Ann. Surg. 134: 614, 1951)

The possible effect of these hormones in prolonging the survival of homografts was studied in experimental animals. Contrary to claims in the literature of that time no such effect was found. (Weisman, P. A., W.C. Quinby, Jr., A. Wight and B. Cannon: The Adrenal Cortical Hormones and

135 mg. percent two days before death. Chart 6A demonstrates that the period of amino-aciduria corresponded roughly to the period of greatest nitrogen negativity. However, amino-aciduria persisted after restoration of positive nitrogen balance, suggesting that the quantitative and qualitative excretion of amino acids was independent of the over-all nitrogen balance.

A 64-year-old woman with a thermal burn of 64% of her body surface, more than half of which was full thickness, died on her tenth hospital day. Amino-aciduria persisted, though subsiding, until the time of death (Chart 7, Case 52-83). In Chart 7A the amino acid excretion again fluctuates independently of over-all nitrogen balance. The terminal reduction of amino acid excretion may have been due to a reduced glomerular filtration.

The Unburned

Chromatography of the preoperative urine specimens of the five patients undergoing surgery revealed a normal pattern in every case. Minor procedures such as hemorrhoidectomy and excision of fissure in ano resulted in little if any deviation from normal. Partial gastrectomy (Chart 8, Case S-3) resulted in somewhat less amino-aciduria than that seen in Chart 5. Hysterectomy and herniorrhaphy resulted in a pattern which lay between that of gastrectomy and hemorrhoidectomy.

The three patients with Cushing's disease showed a greater than normal urinary excretion of amino acids before operation. Chart 9 illustrates the typical findings in one of these cases. The patient was a 42-year-old woman with a ruddy face, abnormal fat distribution, hirsutism, and hypertension. A two-stage, radical, subtotal, adrenalectomy was carried out under cyclopropane anesthesia. The postoperative course was uneventful and the dosage of cortisone was gradually reduced from 200 mg. per day to a daily maintenance dose of 30 mg. over a period of two weeks. After this maintenance

dose had been established, daily urinary collections were carried out for five days. Despite the possible residual effects of surgery, the minimal amino acid excretion is striking. Chart 9A shows the reduction of amino-aciduria in the postoperative phase as well as the over-all nitrogen metabolism.

Discussion

Amino-aciduria accompanied the increased urinary nitrogen loss which follows severe thermal trauma. It was also present after major surgical procedures. It consisted of an increase in both the lower molecular weight, "nonessential" amino acids normally found in urine and the higher molecular weight, "essential" amino acids not usually present. There was some suggestion that both the qualitative and quantitative aspects of the amino-aciduria may be proportional to the severity of the trauma. Patients who were not burned severely enough to endanger their lives and patients undergoing minor surgical procedures showed only minimal amino acid excretion with a preponderance of "nonessential" amino acids. Those dying of their burns or undergoing major surgery had a massive excretion of both "essential" and "nonessential" amino acids.

The actual mechanism of this amino-aciduria is not clear. There are three possibilities. Amino-aciduria may result when elevated blood amino acid levels exceed the renal threshold. With normal blood levels, renal damage and failure of tubular reabsorption may cause amino-aciduria. Finally, amino-aciduria may be seen with normal blood levels and "intact" kidneys in certain "metabolic" disorders -- i.e., "primary amino-aciduria" as in the de Toni-Fanconi syndrome and cystinuria. Although the available data are inadequate to rule out such a mechanism in thermal trauma, a metabolic disorder of this sort seems unlikely. It would seem most likely, then, that the escape of amino acids observed is due to either elevated plasma amino

dressings over corresponding halves of each donor site were removed to favor surface evaporation. Comparisons were made in the healing time of each of the four areas.

More rapid healing was noted in the areas from which the outer dressings were removed than in those left covered. No difference in healing time was attributable to the temperature differential.

B. Shock - Fluid Therapy.

1. Whole Blood versus Plasma as the Colloid Therapy of Choice.

When discussing this problem of burn therapy about which there has been considerable dispute it is essential at the outset to define what we are talking about. Whole blood is badly needed in the therapy of almost all severely burned patients. Anemia develops rapidly in the first weeks in patients with extensive, open, infected wounds. This anemia in our opinion is due to the infection, and repeated replacement of red cells by transfusion is essential to good therapy. The anemia may be apparent well within the first week after injury and continues until the wounds are covered with skin and the infectious process is in abeyance. No one disputes this need for whole blood transfusion. What is in question is the amount of whole blood needed in the fluid therapy of the severely burned patient in the first 48 hours -- the so-called shock phase of burn therapy. The need for whole blood at this time depends logically upon the volume of red cells destroyed by the heat -- those hemolyzed immediately and those sensitized and undergoing subsequent hemolysis.

The question of the colloid solution of choice to be used in the prevention or therapy of burn shock is a critical one both for the Armed Forces and Civilian Defense. If, as some believe, whole blood is an essential part of the initial therapy of the extensively burned patient, then means will have to be found to increase the availability of whole

blood in war areas and in civilian areas threatened with atomic bomb attack in order to treat extensively burned casualties. If, however, plasma is equally or nearly as good as whole blood, then the problem of supply and storage is facilitated. What whole blood is available in an area of disaster could be reserved for those casualties who have suffered true whole blood hemorrhage, the burned patients being treated with plasma or a plasma expander. Whole blood therapy could be postponed until the casualties are evacuated to areas where more whole blood is available. Because of the logistic importance of this question we have continued to push the investigation relating to this problem during the past year.

The history of the development of the different points of view regarding the colloid solution of choice in the therapy of burn shock is worth reviewing. Otherwise it is hard to understand why some take the position that the best therapy is a combination of plasma and electrolyte solutions while others believe that whole blood should be used instead of the plasma or a plasma expander. The difference of opinion stems from a different interpretation of the validity and meaning of measurements made in burned patients and mortality studies in burned animals.

During the early part of World War II a combination of plasma, isotonic electrolyte solution and water emerged as the best fluid therapy for the burned patient in the first two days after injury. Whole blood was to be given in small quantities in the first two days to replace those red cells destroyed by the burn and later in larger quantities as signs of anemia developed. Extensive studies were carried out at this hospital and elsewhere, notably Glasgow, and Birmingham in England, which indicated that at most from 7 to 10% of the red cell mass was destroyed by the heat at the time of the burn. The Evans Blue dye method was the one used for these measurements; this method was then and subsequently has been open to question. (See Moore, Peacock, Blakely, and Cope: Ann. Surg. 124: 811, 1946; and Colebrook, L. et al, Reports of the Burns Unit, Royal Infirmary, Glasgow, 1942-43. Published in 1944.)

In 1944 Moyer, Collier et al (Ann. Surg. 120: 367) reported studies on survival of severely burned dogs indicating that a combination of defibrinated whole blood and an electrolyte solution gave a more prolonged survival than plasma and electrolyte solution. In our own experience the survival time of burned dogs is notoriously variable and we have always wondered whether the results would not be somewhat otherwise if the experiments of Moyer were extended to a larger series.

Near the end of World War II studies made here using radioactive iron tagged red cells to measure the red cell mass confirmed the earlier observations using the less accurate plasma-dye method. (See above

Moore, Peacock, Blakely, and Cope reference.) More recently, 1950, Evans and others have tackled the problem using P-32 tagged red cells. As high as 43% destruction of the red cell mass has been reported.

Puzzled by the discrepancies between our own observations, those of Moyer and Collier, and of Evans, we have re-examined the question using two new approaches.

a. Radioactive Chromium Tagged Red Cells in the Measurement of Red Cell Destruction. Drs. Raker and Rovit have adapted the radioactive chromate method of tagging red cells to the measurement of red cell destruction by the burn. The method was developed by Drs. Sterling and Gray of the Harvard Biophysical Laboratory and offers the best method thus far found for the measurement of the red cell mass in the burned patient. The manuscript by Raker and Rovit describing their trial of the method and its use in experiments with burned dogs was included as Appendix A in the Semi-Annual Progress Report of 30 June 1953. The article is in press in Surgery, Gynecology and Obstetrics.

Raker and Rovit confirm that the chromate ion forms a firm bond with the red cell without destroying the cell. The bond is formed quickly within a half hour. The chromium attaches itself to the globin. If the red cell is hemolyzed by a burn, it sticks to the globin in the plasma. The concentration of chromium in the plasma can thus be used as a measure of hemolysis following a burn in an experimental animal injected with chromium tagged red cells prior to burning. This is the first way in which the chromium tagged cells quantitate the red cell destruction. The second way is by giving a second injection of the tagged cells after the burn and comparing this second volume with the pre-burn volume. It is thus a double check method and good correlation has been observed. In our hands this method has proved better than the radioactive phosphorus method for tagging red cells.

In their experiments with dogs Raker and Rovit found that a burn of 50% of the body surface, of moderate intensity, (immersion in water at 85°C for 30 seconds) gave an average red cell destruction of 8% of the red cell volume before injury. This thermal injury is believed to be comparable to that observed in a chance severe burn of the human being.

When the severity of the burn was increased to a deep cooking burn, (immersion in water at 85°C for 3 minutes) then the destruction of the red cells reached 40% of the red cell volume. This type of severe, deep burn is rarely encountered in the human being. The injury of flash burns, hot water burns, and indeed those of burning clothing rarely penetrate deeply beneath the skin. Only high voltage electrical burns penetrate deeply and these rarely extend to more than one or two extremities.

The percentage of red cell volume destroyed in these experiments is what one would have predicted from the amount of blood circulating in the tissues at the time of burning. Only a small proportion of the body's blood is in the skin or immediately beneath it. Therefore if all of the red cells touched by the burn are destroyed, if the burn is limited to the skin, only a small proportion of the body's red cells will be hemolyzed. If on the other hand the heat reaches into the deeper recesses of the body, it will obviously reach a greater proportion of the red cells and a greater proportion of the total volume will be destroyed.

The red cell volume has been determined by the chromium method in 16 seriously burned patients and in 24 control "normal" persons. In the burned the observations were carried out within the first 24 hours after injury.

Critical evaluation of the observations awaits further work. Evaluation depends upon the assumption made of the volume of red cells before burning. We need more controls and we may have to measure each

surviving burned patient after full recovery. This too might be misleading.

Using the data thus far obtained no evidence has yet been found to indicate that a large fraction of the circulating red cells is destroyed by severe thermal trauma. The results of the patient study are thus in agreement with those of the animal work. Further studies are planned.

b. Blood Viscosity and Blood Flow in the Critical Evaluation of Whole Blood Therapy. The hematocrit of the blood rises as a result of a burn. If whole blood rather than plasma is used as the colloid replacement therapy of the burned patient, the high hematocrit is maintained or further elevated.

It has been demonstrated by Seligman, Blalock and their colleagues that in a variety of experimental conditions the viscosity of the blood varies directly with the hematocrit. This has been found to be true in experimental and human burns by Dr. Quinby. The article describing this work has been published in Surgery and reprints were submitted as technical reports during the period covered by this report. (Quinby, W. C., Jr. and O. Cope: Blood Viscosity and the Whole Blood Therapy of Burns. Surgery 32: 316, 1952.)

It has remained to be shown whether the more viscous blood retards blood flow and endangers the function of the essential unburned organs such as liver, brain, and kidney. Even if the blood flow is retarded by the increased viscosity it must be recognized that the more viscous blood with its high hematocrit has a greater than normal oxygen carrying capacity. Such increased oxygen capacity could permit greater oxygen utilization by the tissues and although the blood flow was reduced the tissues might receive the same amount of oxygen.

This problem of blood flow was first attacked by Dr. Quinby in burned dogs. He tried to measure the blood flow to the brain by quantitating the blood flowing out of the severed internal jugular vein. He did find that the blood flow of the more viscous blood was indeed reduced and that the oxygen utilization from each cc of blood was increased. The results of the experiments varied, however. In some the oxygen utilization was equal, in others reduced.

The variability of the blood flow method employed by Dr. Quinby was so great that we were unable to draw any conclusions from the experiments. This approach has, therefore, been abandoned.

Dr. Wardi has taken up the problem from a different point of view. He is measuring the blood flow through the liver in dogs and burned patients using the method of Dobson. An account of the method used and of the experiments thus far carried out is given by Dr. Wardi in Appendix D. It is fortunate that a method should now be available for observing liver blood flow and that Dr. Dobson is also studying burn trauma in the dog. (Dobson, E. L. and G. F. Warner: Liver Blood Flow Changes in Thermal Injury. To be presented at the Federation Meetings, Atlantic City, 1954.)

Dr. Wardi's results in the dog confirm those of Dobson; liver flow is greatly reduced. He also finds a reduction in the burned patient. These reductions may be due in large part to shunting and in small part to the increased viscosity. More work is needed.

2. Posterior Pituitary Activity in Burn Shock. Dr. Bascom with the collaboration of Dr. Alexander Leaf. Verney has shown that pain and dehydration call forth secretion of the anti-diuretic hormone of the posterior pituitary. Both pain and dehydration occur in the burned patient. The finding in our extensively burned patients of continued high specific gravity urine suggests that the posterior pituitary may well play a part in the compensation

evidence of renal failure. All had received a high sodium intake intravenously for the first 48 hours. Seventy-five cc plasma and 75 cc saline solution had been given for each 1% of body surface burned in the first 24 hours and one half that quantity in the second 24 hours. Water and glucose had been given in addition for kidney function. In the subsequent days the sodium intake had been moderate to low. In spite of a positive sodium balance the serum sodium concentration dropped promptly below normal to the 120-135 meq/l range and stayed low for a few to several weeks. Additional sodium chloride intake did not raise the concentration. This lowering of the serum sodium concentration we believe to be part of the normal, "healthy" response of the severely burned patient.

Six patients had renal damage and died of renal failure. Three were given the above routine therapy. The serum sodium levels of all rose to above normal. Sodium poured out in the exudate reducing the sodium balance to slightly positive.

The other three with renal failure received less sodium in therapy because the renal damage was recognized promptly and therapy restrained. Their serum sodium levels fell promptly to approximately 120 meq/l and stayed there. They lost more sodium in the exudate than the 24 patients with functioning kidneys but less than the three who received higher intakes. They were in approximate sodium balance. On the 12th day one received 3% sodium chloride solution and his serum sodium rose rapidly to 160 meq. This salt was given him because of a fear that the level of 120 meq was deleterious. At that time he was clear mentally and considered to be weathering his renal damage satisfactorily. After the hypertonic salt solution he deteriorated and died in 36 hours.

The balance and exudate studies indicate that with renal impairment the wounds act as a sluiceway for sodium. Graphs of the serum levels of these three groups are given as Appendix G.

Raker and Minor are also studying the sodium and electrolyte problem from the angle of cell penetration and cell loss. In dogs they are measuring the sodium space and the electrolyte concentration of unburned as well as burned tissues using radioactive sodium and chemical methods. The dogs are being treated with high, medium and low sodium intakes.

4. Do Pressure Dressings Effectively Diminish the Need for Fluid Therapy? In World War II it was an accepted principle of burn therapy that pressure dressings should be applied to the head and extremities of the burned patient in order to diminish the fluid needed as therapy or in prevention of burn shock. This attitude had many early backers and seemed well supported by experimental evidence of Siler and Reid, Glenn and Drinker, and others. The impression of many clinicians fell in with the experimental evidence and the principle became accepted.

With the recent swing toward open therapy of the burn wound initiated by Wallace, Blocker and Pulaski, the possible value of the pressure dressing in sparing fluid seems to have been lost sight of. Has this been wise? Are we forgetting something important? In seeking simplicity of the care of the wound are we adding to our troubles in the fluid therapy of the burned patient?

Two years ago Dr. Vilain, a French research fellow in plastic surgery, was assigned this problem of testing the efficacy of pressure dressings. The problem proved more difficult than anticipated for him and a little more than a year ago Dr. Wight joined to help him. Dr. Vilain returned to France three months later and Dr. Wight has continued with the observations. They are not yet completed to her satisfaction and it is hoped that during this next six months she will be able to devote additional time to complete them.

The observations may be tentatively summarized as follows. It is probable that a properly applied pressure dressing or a tight fitting plaster

cast does diminish the loss of fluid from the plasma into the burn wound. The decrease in loss is slight, however, and therefore the experimental method needed to measure it has to be refined.

From the point of view of the clinical therapy of the burned patient it can probably be said that proper pressure dressings applied to burns of the extremities and the head do diminish slightly the volume of replacement therapy needed in the prevention or treatment of burn shock. The volume spared is slight and not too much is being sacrificed in the fluid therapy of the patient when the burn wound is treated by the open method. This is certainly true when adequate supplies of plasma or a plasma expander are available for the therapy and when the veins available are adequate.

C. Intermediary Metabolism.

1. The Elevation of the Metabolic Rate in the Burned Patient.

The metabolic rate (oxygen consumption) of the severely burned patient has been studied and found to be elevated to levels comparable to those of severe hyperthyroidism. The rise in rate is early and abrupt and the rate slowly subsides as wound healing takes place. Measurements of thyroid function (protein-bound iodine and radioactive iodine turnover in the thyroid gland) indicate that thyroid function remains within the normal range and that the thyroid hormone is not responsible. The outburst of adrenal cortical activity is also excluded as etiologic since the metabolic rate and radioactive iodine turnover are slightly depressed in Cushing's disease. We have the hunch it is related to wound metabolism. It certainly explains the rapid wasting which these patients suffer. This study has been published during the period covered by this report and reprints have already been distributed. (Cope, O., G. L. Nardi, M. Quijano, R. L. Rovit, J. B. Stanbury and A. Wight: Metabolic Rate and Thyroid Function Following Acute Thermal Trauma in Man. Ann. Surg. 137: 165, 1953) We have

turned to measuring metabolic rate and thyroid function in other conditions of trauma including patients with major fractures and severe chronic infections.

2. The Meaning of the Flood Tide of the Eosinophils in the Severely Burned Patient. In the report of a year ago we described the varying course of the eosinophil level in the blood following a burn injury emphasizing the serious prognostic significance of a continued low eosinophil count and the good omen of flood tide of eosinophils in the severely damaged patient responding well to his trauma. This work has now been published and reprints forwarded as technical reports during the period covered by this report. (Wight, A., J. W. Baker, W. R. Merrington and O. Cope: The Ebb and Flood of the Eosinophils in the Burned Patient and Their Use in the Clinical Management. Ann. Surg. 137: 175, 1953)

The published report described nine patients with continued low eosinophil counts who died as a consequence of their burn injury. We felt reasonably certain of our findings in that group. In the group with the flood tide of eosinophils later during the course of the grafting and healing of their wounds, there were only five patients. The range of eosinophils was considerable and we felt that further observations were indicated, first to substantiate the flood tide as a phenomenon and second to find out its meaning.

Accordingly we have continued to observe the eosinophil level of the blood in severely burned patients. We can report that the flood tide is indeed a phenomenon frequently encountered in the severely burned. We enclose a chart of a case encountered in these last six months. (See next page) The clinical course of this patient is described in the legend. An article has also appeared by Dr. Sevitt, from the Medical Research Council Burns Unit in Birmingham, England, describing the identical phenomenon. His article appeared while we were assembling our own data. Unfortunately we were not aware of the article and therefore did not refer to his work in our report.

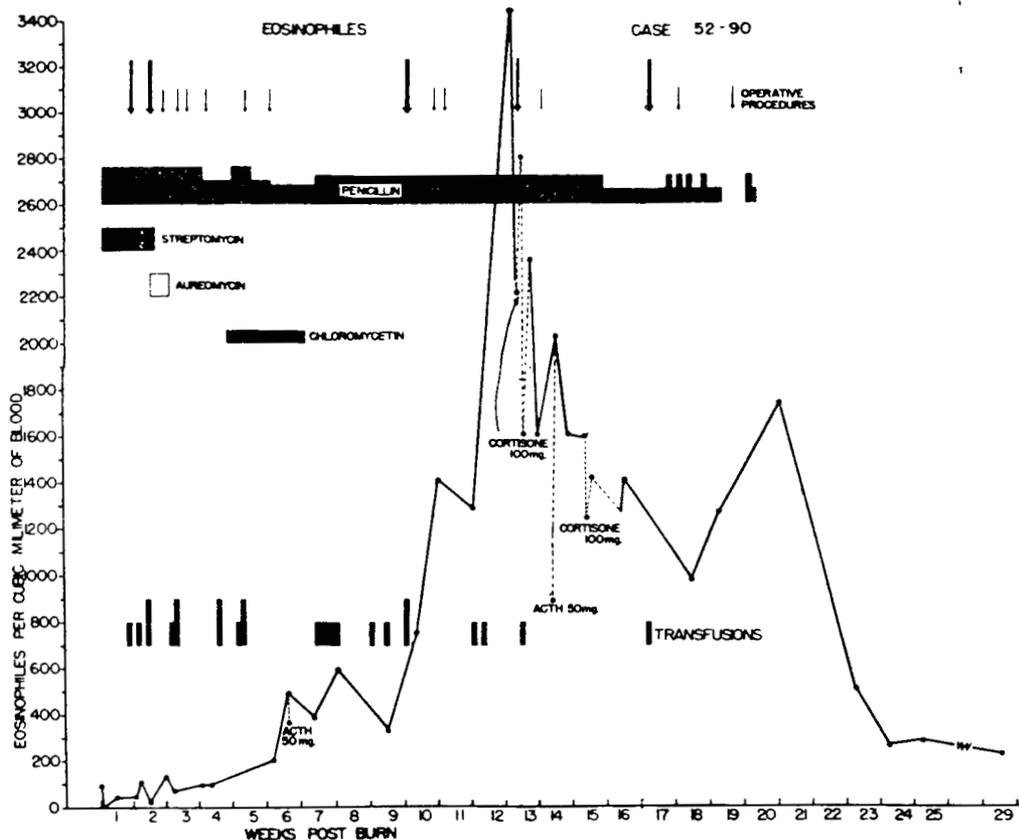


CHART ILLUSTRATING THE COURSE OF THE BLOOD EOSINOPHILES IN A PATIENT WITH DEEP BURNS OF MODERATE EXTENT. A 26 year old white male suffered a 20% third degree burn involving the entire circumference of both legs from the ankles to above the knees. The eosinophiles fell to almost zero shortly after the trauma. Excision and grafting of the burned areas was carried out on the sixth and eleventh days, and the patient responded well in the early period with initial take of the grafts. Supportive therapy included several whole blood transfusions. Gradually the grafts melted, apparently due to infection, and his condition deteriorated through the sixth week. There was only a moderate rise in his eosinophile count. In the seventh week he was given a daily transfusion for five days with marked improvement in his appearance and with cleaning up of the denuded areas. The third major operative procedure was carried out in the tenth week as his eosinophiles were rising at a rapid rate. From this date he did well clinically although a long time was required to clear up the infection of the burned areas and get them covered with skin. After reaching the peak of 3334 eos/cu.mm. in the thirteenth week, the count slowly fell as the patient improved, until it reached the normal level in the twenty-fourth week when he was nearly ready to go home. ACTH and oral cortisone given during the flood tide were followed by a significant drop in the eosinophile level.

Our interest has extended beyond confirmation to try to find out the meaning of the flood tide. We have been able to find that the elevated eosinophil count is still suppressed by cortisone or ACTH. It therefore remains under potential control of the adrenal gland. The flood tide is present throughout a period when Drs. Nathanson and Engel are able to demonstrate abnormally large quantities of the corticoids in the urine. The flood tide occurs therefore presumably when the adrenal is still hyperactive. Some other influence has become dominant. In considering the possibilities it seems logical to us to believe that the flood tide is related to the infection. Because the patient is doing well during the flood tide it represents a good omen and perhaps is related to a good immune body response of the patient to the infecting organisms. We have no factual evidence to support this concept -- only armchair reasoning.

3. Pattern of Urinary Steroids. Drs. Engel and Nathanson. Drs. Lewis Engel and Ira T. Nathanson have continued their studies of the urinary steroid excretion in collaboration with us. The emphasis during this year has been in patients with adrenal cortical disease. Four patients with the Cushing's type of adrenal cortical disease and two with adrenal cortical virilism have been studied both before and after operation upon the adrenal glands with reduction in adrenocortical activity. It is believed that the findings in these patients will prove an interesting contrast to the steroid pattern of the burned patient. In addition four patients have been studied before and after total adrenalectomy for metastatic carcinoma of the breast. A comparison of the various clinical states associated with changes in adrenal cortical function should throw some light on the pattern of adrenal over-activity following burn trauma.

4. Intermediary Nitrogen Metabolism. Dr. Nardi. This part of our program has become a dominant and exciting one. Dr. Nardi has discovered a break in the wall of mystery enclosing the upset in nitrogen metabolism which follows burn trauma. The background of this project is as follows.

In 1945-46 Drs. Mathanson and Hilda Wilson, while checking the completeness of 24 hour urine collections in our burned patients, analyzed the urine for both creatine and creatinine. They found that both were excreted in large quantities, indicating an important derangement in nitrogen metabolism (unpublished data). Walker and others have subsequently reported similar findings.

Dr. Nardi has widened the study by analyzing the urinary excretion pattern of more nitrogen fractions, by measuring the blood serum level of the several amino acids and by the disappearance rate of S-35 tagged methionine. He has found an outpouring of a large quantity of amino acids in the urine. The excretion of the non-essential amino acids normally present is increased. There are in addition other non-essential and essential amino acids put out in large quantities. The excretion of both non-essential and essential amino acids is larger than would be expected from the increase in total nitrogen. Therefore the amino acid concentration is abnormally great. This work has been written up and accepted for publication in the Journal of Clinical Investigation. The manuscript is appended as Appendix A.

In addition Dr. Nardi has studied the excretion pattern of the amino acids in patients with Cushing's disease and in patients before and after a variety of major operations. In the patients with Cushing's disease there is also an outpouring of a variety of amino acids which disappears after subtotal adrenalectomy. Following various operations the excretion is

increased qualitatively and quantitatively. The variety and amount of amino acids is proportional to the magnitude of the surgery. In this latter group at least he suspects that the findings are the result of the failure of tubular resorption. This work has also been accepted for publication in Surgery (See Appendix B).

Paper chromatography has been adapted to the measurement of free amino acids in the blood plasma and studies are in progress in Cushing's disease, other surgical conditions and in burned patients. An article describing an original method devised in this laboratory for the chromatography of free amino acids in blood has been accepted for publication by the Journal of Laboratory and Clinical Medicine and is to appear in the May, 1954 issue. (See Appendix C)

Partition studies of the total nitrogen excretion in the urine and of the loss in wound exudate are under study in the burned patient. The protein turnover rate is being studied by Dr. Nardi in the severely burned and in patients with Cushing's disease. The experience to date in these two areas is recounted under Plans-Immediate. As with the other nitrogen studies they are directed at elucidating the role of the adrenal cortex in the metabolic response to trauma.

D. Acute Dehydration, Potassium Deficiency and Metabolic Derangement Following Perforation of a Peptic Ulcer.

The acute effects of the perforation of a peptic ulcer on water and electrolyte distribution and nitrogen balance have been studied in 24 patients. The assembling of the data has been completed, the conclusions drawn, and written up in four manuscripts. These will shortly be submitted for publication. For the sake of brevity of this Annual Report only the first manuscript is appended (see Appendix F). This first manuscript gives the scope and character of the study. The reason for the study is given in the introduction.

It describes the evidence for the acute dehydration which occurs as a result of the chemical burn of the peritoneum.

The second manuscript describes the shifts in potassium and phosphorus, the evidence for loss of these intracellular electrolytes. In those patients receiving no parenteral potassium therapy there is a decline in the serum potassium level to below normal. The decline is concomitant with an outpouring of potassium in the urine and a negative potassium balance. The drop in serum potassium is prevented by including potassium in the fluid therapy during the shock and postoperative peritonitis phases. It is probable, though not proven by our cases, that this acute potassium loss and low serum potassium level contributes to the continued high mortality in this condition. Therapy in the future should include parenteral potassium. It is of note that the burned patient does not suffer as large an acute potassium loss in the urine and does not exhibit a drop in serum potassium level.

The third manuscript describes the shifts in sodium and chloride. An interpretation of the sodium and thiocyanate spaces is given.

The fourth manuscript recounts the nitrogen balance. Maintenance of potassium balance does not affect the nitrogen loss. A comprehensive program of therapy is described.

Since a significant difference has been found in the metabolic response of these patients as compared to that of burned patients, study of other forms of trauma, fractures and intestinal injuries, is indicated.

PLANS FOR THE FUTURE

I. Immediate

A. Wound Healing.

1. The Effect of Temperature and Humidity on the Rate of Wound Healing. This project bearing on the open versus closed method of treating burn wounds will be continued as patients become available. The studies have two strong points. First they are being carried out in the human being. Second, they are of fundamental character and should prove of interest to the management of other kinds of wounds.

B. Shock - Fluid Therapy.

1. Whole Blood versus Plasma as the Colloid Therapy of Choice.

a. Radioactive Chromium Tagged Red Cells in the Measurement of Red Cell Destruction. Drs. Baker and Minor. Extensively burned patients will continue to be studied for evidence of red cell destruction by the burn, using the radioactive chromium tagged method. This project is of practical importance to both the Armed Services and Civilian Defense. (See Abstract Section B,1,a of this Report and Appendix A of Semi-Annual Progress Report of 30 June 1953.)

b. Blood Viscosity and Blood Flow in the Critical Evaluation of Whole Blood Therapy. Dr. Nardi will continue his studies of liver blood flow in various clinical states including burn shock. This study is significant not only to the question of the use of whole blood in burns with its consequent rise in hematocrit and viscosity but it should also yield information upon the role of the liver.

2. Posterior Pituitary Activity in Burn Shock. Drs. Bascom and Leaf. It is planned to continue the study of the diuretic mechanism in burn shock and the role of the posterior pituitary with its anti-diuretic hormone.

We do not as yet have enough information to know to what extent this project will be carried. We are still in the pilot stages. Our earlier interest in renal blockage by an excess of calcium has been absorbed by this project.

3. Edema Volume and Need for Sodium in the Shock Therapy of the Burned Patient. Drs. Baker and Minor. This is a project of immediate concern to therapy but because of its difficult fundamental character involving cell milieu and intracellular balance it is also a long range one(See Appendix G).

4. Do Pressure Dressings Effectively Diminish the Need for Fluid Therapy. Dr. Wight. At the moment there seem to be more pressing studies than this one. Although not satisfactorily answered, our observations to date suggest that any advantage accruing from the use of pressure dressings is slight. To be certain of this will require a lot more work and other studies are more pressing. It will probably prove wise to come back to it later since it should be answered securely.

C. Intermediary Metabolism.

1. Metabolic Rate and Thyroid Function in Trauma. We plan to continue this interest in other fields of trauma including fractures and chronic infections.

2. The Meaning of the Flood Tide of the Eosinophils in the Severely Burned Patient. This study too is to be continued and spread to other forms of trauma and particularly to patients with severe chronic sepsis when such are encountered. It involves the relation of the adrenal cortex not only to stress of trauma but also to the immune reaction.

3. Pattern of Urinary Steroids. Drs. Engel and Nathanson. By adapting chromatography to the final step of 17-ketosteroid analysis Dr. Engel has succeeded in dividing that group of steroids into some 15 compounds. The curve of excretion of those separate compounds will now be studied in the urine

of burned patients, the gross or collective curve having been adequately described. One of the chemical technicians is to be transferred to full time in this work. Later the other two classes of steroids will be similarly analyzed.

In the same patients, a pilot study is to be made by a psychiatrist of the day to day mental attitudes. Severely burned patients not infrequently develop a psychotic-like reaction after the first week or 10 days, reminiscent of the psychosis encountered in patients under ACTH or cortisone therapy. The steroid excretion will be compared with the mental pattern. It is hoped in this way to learn something about why burned patients are such difficult behavior problems to their nurses and doctors. This portion of the project will be in collaboration with the Unit of Child Psychiatry of this hospital.

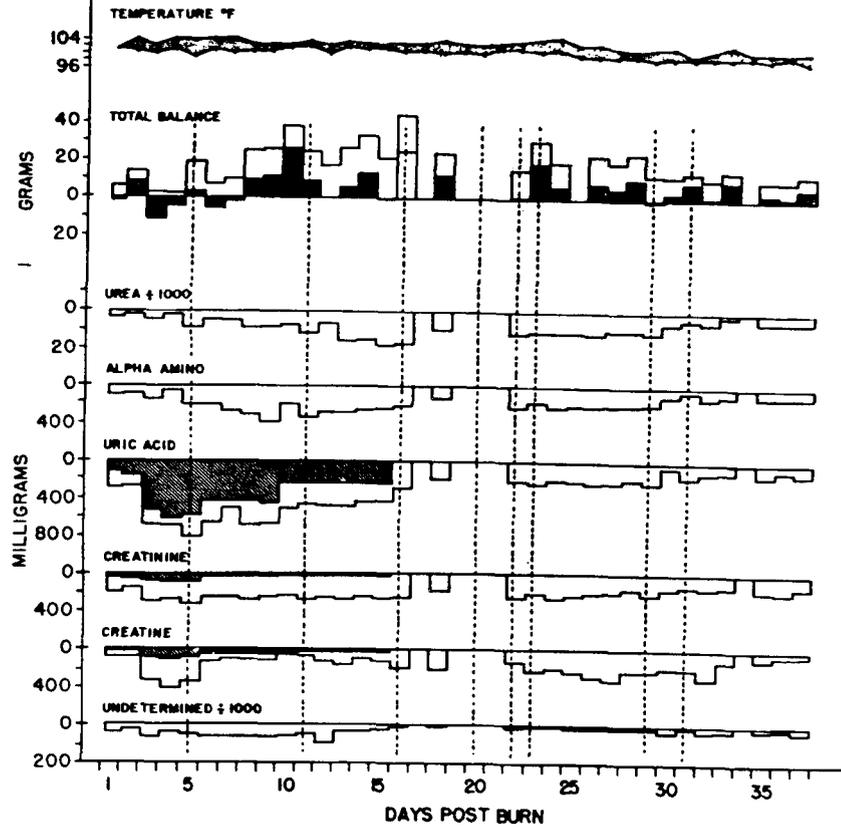
4. Intermediary Nitrogen Metabolism. Dr. Nardi. Dr. Nardi is pushing his nitrogen studies as fast as conditions permit and plans so to continue. (See also Long Range)

To determine the significance of the negative nitrogen balance, a dissection of the nitrogen excretion of badly burned patients has been undertaken. Each individual metabolite has its own role in the body physiology. The loss in the wound exudate as well as urine has been measured. The work is illustrated in the accompanying chart termed "Nitrogen Partition" of one of the patients.

Patient 53-46 was a 9 year old boy who suffered third degree burns of approximately 40% of the body surface. A total balance of nitrogen was carried out in a conventional fashion, intake being carefully calculated and weighed and 24 hour urine collections being subjected to a Kjeldhal analysis for nitrogen. This type of determination was carried out for a period of 37 days. Simultaneous studies were carried out on the burn exudate for the first

NITROGEN PARTITION

BURNED PATIENT 53-46



15 days by extracting and analyzing the dressings. As can be seen in the total balance, there is an early phase of negative balance in the first few days following the burns and of considerable significance from a therapeutic point of view is the fact that the negative nitrogen balance can be minimized by adequate nitrogen intake.

In order to carry out the above-mentioned dissection of the nitrogen excretion, determinations for urea, alpha amino nitrogen, uric acid, creatinine, and creatine were carried out on aliquots of the urine and exudate. The difference between the sum of these values and the total nitrogen excretion was termed "undetermined nitrogen" and plotted as such.

Several interesting and pertinent observations result from analysis of this data. In the early days the undetermined nitrogen makes up by far the greatest component of nitrogen excretion. It is believed that a great part of this undetermined nitrogen may be polypeptide nitrogen and experiments are now under way to confirm this suspicion.

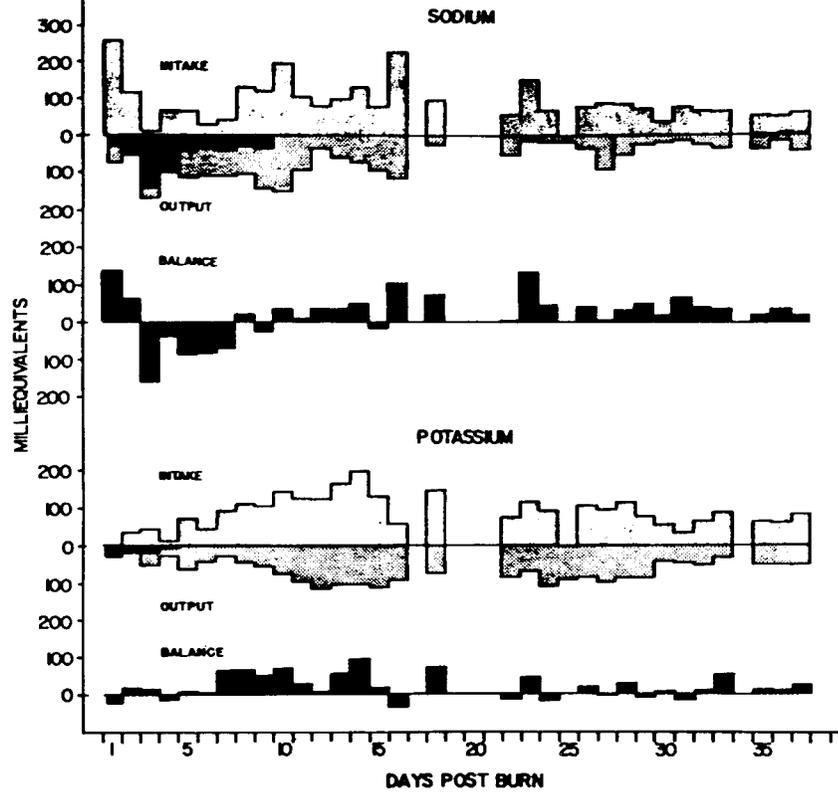
Urea, as might be expected, forms the second largest component of the nitrogen excretion. However, this does not seem to reach a stable value until after a week or more and then remains at relatively constant levels.

Our initial observations of the independence of the amino acid excretion seem confirmed by this quantitative study in which it can easily be seen that the quantitative alpha amino excretion reaches a peak value considerably after the overall nitrogen excretion has reached its peak value. Amino aciduria seems to continue long after the restoration of an overall total nitrogen balance.

The shaded areas of uric acid, creatinine, and creatine represent the amount of these compounds present in the exudate nitrogen. The large quantities of these compounds present again emphasizes the necessity of exudate analyses before an overall balance can be determined for any burned

ELECTROLYTE BALANCE

BURNED PATIENT 53-46



patient, i.e., losses from the burn wound may make up a major part of metabolite excretion in the burned patient. This latter point is again emphasized by the quantitative studies of electrolytes in this same patient (second Chart) in which it can be seen that the major portion of sodium excretion in the first 10 days after burning is to be found in the exudate rather than in the urine.

In the recent study of the elevated metabolic rate and thyroid function in the severely burned patients it was postulated that the increased oxygen consumption was due to the metabolic demands of the burn wound. To help elucidate this metabolic acceleration it was decided to determine the protein turnover rate in patients who had suffered severe burns and in patients with Cushing's disease, the latter to evaluate the role of adrenal hyperfunction.

S-35 labeled methionine represents a research tool which can be used safely in the human subject for the evaluation of protein metabolism. The incorporation of this material into plasma proteins in normal individuals occurs at a predictable rate. This is equally true of its later disappearance from plasma proteins. Since the S-35 concentration in the plasma protein-free filtrate reaches levels too low to permit measurement within 48 hours of S-35 administration, one may assume that at this time (48 hours) all S-35 which has not been excreted is incorporated in various organs and tissues. Hence the decreasing concentration of S-35 in plasma protein after the initial 48 hour period may be regarded as an index of the rate of catabolism of plasma protein. The half life of plasma protein in normal individuals measured in this fashion is from 24 to 32 days. (Kinsell, W. L. et al: J. Clin. Invest. 29: 238, 1950)

Two patients with Cushing's disease were injected intravenously with 50 microcuries of S-35 methionine. Daily blood samples were drawn after 48 hours and assayed for radioactivity. The protein half-life of both these patients was 12.5 days, suggesting that adrenal hyperfunction per se increases

protein catabolism. These findings are in accord with that of Kinsell et al in their one patient with Cushing's disease.

Two severely burned patients were studied in a similar fashion. Both patients had severe burns of 40-50% of their body surface. Injection was carried out during the sixth post-burn week at which time the wounds were only partially healed. Protein half lives of 8.1 days and 11 days were found, indicating a protein turnover even more rapid than that associated with the adrenal hyperactivity of the degree in those patients with Cushing's disease.

These findings also lend support to the concept that the increased oxygen consumption observed in severely burned patients is a manifestation of the intense metabolic activity resulting from the body's efforts to repair the burned wound. It is obvious that many further observations are needed, observations in more patients and in the burned patients at varying intervals after injury including during the convalescence.

II. Long Range

Surgical conditions associated with trauma, depletion and repair should continue to be rewarding fields of investigation. Two themes have dominated our interest in the past and will continue to do so in the future. These themes are the intracellular phase of metabolism and the endocrine component in the metabolic response to these surgical conditions.

Clinical research has advanced only in recent years to the point where it can attack the intracellular phase of metabolism. It had its advent with World War II; progress has been slow because methodology is still largely dependent upon indirect measurement. The history of the investigation of shock illuminates well the slow rate of progress of investigation in this field. During World War I investigators determined that maintenance of the circulating blood volume was the essential of the therapy of shock. A colloid solution was found necessary since the intravenous injection of a salt solution without

colloid gave only a temporary rise in blood pressure. That the salt solution seeped out through the capillaries was observed, but where it went was little understood. In the period between the two World Wars the extracellular fluid space was defined. It was into this space that the salt solution seeped. It was not until World War II that clinical investigators became aware of the intracellular space. Although the biochemist had for many years pointed out the chemical significance of the difference between the intra- and extracellular phases, there was a long latent period between their observations and the clinicians' appreciation of their possible meaning to therapy.

Our understanding of the cell membrane and its meaning to therapy is still hazy and full of conjecture. Methods of attack in investigation are still largely dependent upon measurements made in the extracellular phase. New approaches are needed. In our program we are constantly evaluating new approaches such as the adaptation of isotope techniques and tissue biopsies. Interpretation of biopsy data remains dependent upon assumption and calculation but they are nearer to direct measurements of intracellular phases than the analysis of blood plasma, lymph and measurements of total balance exchanges. We look toward enlarging our use of tissue biopsies, of undamaged as well as damaged tissues, in our future program.

The endocrine theme of our studies is based upon the long known and firmly established fact that the balance of the endocrine system is disturbed by trauma and other diseases such as acute infection. Since the endocrine system has a dominant influence in the metabolism of many organs and tissues it has seemed wise to compare the abnormal metabolism of spontaneous endocrine diseases with that following trauma and other surgical states associated with depletion and wound healing problems. We feel reasonably sure on our present evidence that the whole upset of metabolism following trauma is not satisfactorily explained by the adrenal cortex alone. It is true there are many similarities

between the endocrine response to trauma and spontaneous Cushing's disease, for the outstanding feature of Cushing's disease in our minds is a slow, cellular depletion. On the other hand there are several observations, such as the high oxygen consumption of the burned patient which do not fit satisfactorily with an outburst of adrenal cortical activity alone. We therefore should plan to continue to investigate spontaneously occurring endocrine situations associated with tissue depletion. We will concentrate on patients with Cushing's disease as such become available. The observations will be compared with those made in patients following trauma, infections, etc.

The study of the burned patient will be continued during these next several months. There is much to be done in burns, but there is a practical reason for shifting to other fields of trauma or to other diseases if the particular object in view is equally well satisfied. Investigation in the field of burns is costly in terms of personnel. It is impossible to do metabolic observations on patients without complete supervision of the patient care. The carrying out of this patient care robs time of the investigator which might otherwise be applied to his researches. Burned patients demand an enormous amount of professional attention. During the year 1953 the research personnel of this project supervised the care of 115 burned patients.

The program of the future envisages continuation of investigation in the same areas of the burned patient -- the wound, shock-fluid therapy and intermediary metabolism. Wounds are a part of surgical disease and observations of wound healing in the human being and careful selection of experiments in animals are an essential part of the investigation of trauma.

The areas of fluid therapy and intermediary metabolism overlap. Physiologically one cannot separate nitrogen metabolism from electrolyte and water balances. They have been separately defined for simplicity of presentation.

Studies of the distribution of sodium and potassium will be pursued. We hope eventually to master a more rapid technique for the measurement of magnesium and calcium. Dr. Wardi will continue his studies of nitrogen metabolism.

The responsible investigator has repeatedly expressed his uneasiness and dissatisfaction that there is no one in our investigative unit whose primary interest is in the infectious aspect of trauma, no one with special competence in infectious disease. For a year and a half we had a qualified bacteriology technician, but her skilled services were not sufficient to manage a critical research program. Without research in infection our program may be lopsided; we run the danger of assuming that the observed changes may be due to an endocrine disturbance when in reality they are basically related to infection.

Fifteen years ago extensively burned patients died within the first 24 hours, presumably from inadequate fluid therapy. In the intervening years sufficient has been learned about the management of the dehydration and fluid imbalance to the extent that such patients survive the first few days after their injury provided therapy is started promptly. We are now confronted with the spectacle, therefore, of extensively and deeply burned patients surviving several days only to die in spite of all efforts. These deaths appear to be due to infection. Our use of anti-bacterial chemotherapy, although it has achieved much, is not able to obliterate bacterial growth, and infection mounts in the widespread wounds. This is a clinical impression, not known fact, and the only way to unravel the problem is by continued research both into the abnormalities of cellular metabolism and renewed research into the infectious disease aspects. As far as our own planning is concerned, should somebody join our staff with a special interest and competence in the field of infection it would be considered wise for him to renew the investigation of the infectious aspect of the burn problem.

REPORTS AND PUBLICATIONS

A. Articles published and reprints submitted during the period of this report:

1. Quinby, W. C., Jr. and O. Cope: Blood Viscosity and the Whole Blood Therapy of Burns. *Surgery* 32: 316, 1952.
2. Wight, A., J. W. Raker, W. R. Merrington and O. Cope: The Ebb and Flood of the Eosinophils in the Burned Patient and Their Use in the Clinical Management. *Ann. Surg.* 137: 175, 1953.
3. Cope, O., G. L. Nardi, M. Quijano, R. L. Rovit, J. B. Stanbury and A. Wight: Metabolic Rate and Thyroid Function Following Acute Thermal Trauma in Man. *Ann. Surg.* 137: 165, 1953.
4. Nardi, G. L.: Radioactive Measurement of Proteolytic Activity. *Science* 118: 299, 1953. (Manuscript appended to Semi-Annual Progress Report of 30 June 1953 as Appendix E.)

B. Manuscripts in press or submitted for publication:

1. Raker, J. W. and R. L. Rovit: The Acute Red Blood Cell Destruction Following Severe Thermal Trauma in Dogs; Based on the Use of Radioactive Chromate Tagged Red Blood Cells. In press. *Surgery, Gynecology & Obstetrics*. Manuscript appended to Semi-Annual Progress Report of 30 June 1953 as Appendix A.
2. Nardi, G. L.: "Essential" and "Non Essential" Amino Acids in the Urine of Severely Burned Patients. In press. *Journal of Clinical Investigation*. Manuscript appended as Appendix C.
3. Nardi, G. L.: Urinary Loss of Amino Acids after Surgery. Accepted for publication by *Surgery*. Manuscript appended to Semi-Annual Progress Report of 30 June 1953 as Appendix D.
4. Gordon, Spencer, Jr. and G. L. Nardi: Paper Chromatography of Free Amino Acids in Blood Plasma. In press. *J. Lab. and Clin. Med.*

5. Cope, O., J. F. Hopkirk and A. Wight: The Physiologic Derangements and Deficits Imperiling the Patient with a Perforated Peptic Ulcer. I. The Dehydration and Fluid Shifts. To be submitted to Archives of Surgery.
6. Wight, A., J. F. Hopkirk, E. DeMuylder and O. Cope: The Physiologic Derangements and Deficits Imperiling the Patient with a Perforated Peptic Ulcer. II. Derangements and Shifts of Intracellular Electrolytes. The Need for Potassium. To be submitted to Archives of Surgery.
7. Wight, A., S. Taylor, C. L. Minor, W. Lohnes, J. F. Hopkirk and O. Cope: The Physiologic Derangements and Deficits Imperiling the Patient with a Perforated Peptic Ulcer. III. Derangements and Shifts of Sodium and Chloride. To be submitted to Archives of Surgery.
8. Cope, O., A. Wight and J. F. Hopkirk: The Physiologic Derangements and Deficits Imperiling the Patient with a Perforated Peptic Ulcer. IV. Derangements of Nitrogen Metabolism and the Nitrogen Deficit. The Comprehensive Plan of Therapy. To be submitted to Archives of Surgery.

C. Manuscripts in preparation:

1. Cope, O. and J. W. Raker: Cushing's Disease: The Surgical Experience in Treating 45 Cases.

"ESSENTIAL" AND "NONESSENTIAL" AMINO ACIDS
IN THE URINE OF SEVERELY BURNED PATIENTS

George L. Nardi, M.D.

From the Department of Surgery of the Harvard Medical School at the
Massachusetts General Hospital, Boston, Massachusetts

These studies were aided by financial support from Departments of the
Army and Navy. NR 114-198

Introduction

The negative nitrogen balance of thermal trauma is a well recognized phenomenon.^{1,2} The meaning and significance of this to body metabolism are poorly understood. Partition of urinary nitrogen may assist in interpreting the significance of nitrogen loss. Walker³ and Taylor et al⁴ found an increase in nonprotein urinary nitrogen of burned patients. This was chiefly due to undertermined nitrogen, the latter defined as the difference between the total nonprotein nitrogen and the sum of urea, uric acid, creatinine, and alpha amino nitrogen. To analyze this partition further we have studied the urinary amino acid excretion of severely burned patients. Amino-aciduria has been found after severe thermal trauma. A similar amino-aciduria has been observed after comparable surgical trauma, suggesting that the phenomenon is nonspecific. Similar studies on patients with hypercorticism (Cushing's disease) suggest that the adrenal gland may be operative in the amino-aciduria of thermal trauma.

Materials and Methods

The paper chromatographic technic described by Martin and Synge,⁵ Consden et al⁶, and Dent⁷ was utilized to study the urinary excretion of free amino acids. Hydrolysis of urine was not carried out, and so the role of peptiduria cannot be evaluated.

Aliquots of 24-hour urine collections were analyzed for nitrogen (Kjeldahl) and volumes corresponding to 250 micrograms of total nitrogen were chromatographed two-dimensionally. The first solvent consisted of phenol and water; and the second, of butanol, propionic acid, and water.⁸ The papers were developed by spraying lightly with 0.1% ninhydrin in ethanol.

Using this technic, urine of normal people is found to contain mainly glycine and alanine with occasional traces of other "nonessential" amino acids.

In general, only the lower molecular weight, "nonessential" amino acids are found in urine.^{9,10,11}

Five members of our laboratory personnel served as "normals." Chart 1 represents the amino acid pattern of the person in this group showing the greatest excretion of amino acids. It also illustrates our charting conventions. The amino acids have been arbitrarily classified as "essential" and "nonessential" in terms of the latest available information regarding the necessity of their presence in the diet of normal adult human beings in order to maintain positive nitrogen balance.¹² Within both groups the amino acids have been arranged in order of increasing molecular weight.

The Severely Burned

Six severely burned patients were studied. All were brought to the hospital shortly after injury except for Case No. 52-51, who was transferred from another hospital 48 hours after injury. All had suffered extensive thermal trauma involving from 15 to 65 percent of the total body surface.

All patients were in good general health prior to being burned. Treatment was similar in each instance. Plasma and blood were used for colloid replacement according to a standard formula.¹³ Penicillin and streptomycin were administered in every case. All the burned areas were treated with occlusive dressings. Administration and calculation of oral intake were supervised by a special dietitian. Twenty-four-hour urine samples were collected in bottles containing thymol and toluol by means of an indwelling catheter. Aliquots were used for analyses.

The Unburned

Similar observations were done on patients undergoing conventional surgical procedures. In order of increasing magnitude these procedures were: excision

NORMAL		WEAK	DAYS				
ESSENTIAL AMINO ACIDS		MOLEC. WEIGHT	1	2	3	4	5
THREONINE	105						WEAK
VALINE	117						
LEUCINE	131						
ISOLEUCINE	131						
LYSINE	146		STRONG				
METHIONINE	149						
PHENYLALANINE	165						
TRYPTOPHAN	204						
TOTAL			1				1
NON-ESSENTIAL AMINO ACIDS							
GLYCINE	75		WEAK	STRONG	WEAK		
ALANINE	89			STRONG			
SERINE	105		WEAK				WEAK
PROLINE	115						
HYDROXYPROLINE	131						
ASPARTIC ACID	133						
HISTIDINE	155				WEAK		
ARGININE	174						
TYROSINE	181						
GLUTAMIC ACID	183						
CYSTINE	240						
TOTAL			2	2	4	2	3
UNKNOWN							
TAURINE							
GLUTAMINE							
TOTAL			2	3	4	2	4

CHART I

of fissure in ano, hemorrhoidectomy, herniorrhaphy, total hysterectomy, and subtotal gastrectomy. All operations were elective and all patients in good general and nutritional condition. Gas-oxygen-ether anesthesia was used in all but the first two cases, where spinal anesthesia was utilized. Post-operatively, saline, glucose, and water were administered intravenously until the patient's oral intake was adequate.

In an attempt to evaluate the role of the adrenal glands, three patients with hypercorticism due to fullblown Cushing's disease were studied. Twenty-four-hour urine collections were carried out before and after radical subtotal adrenalectomy. A minimum of fourteen days was permitted to elapse after surgery so that any effects due to surgical trauma per se (as seen in the previous group) might be minimized.

Results

The Burned

The findings in the burned patients are reported in order of increasing severity. There are two charts for some patients. The first represents the amino acid excretion patterned after Chart 1; the second corresponding chart, suffixed by the letter A, shows the simultaneous over-all nitrogen balance and gross amino-aciduria for the same patient. Since volumes of urine containing equal amounts of nitrogen were chromatographed daily, the significance of the "A" chart becomes obvious. If the amino-aciduria represents a constant percentage of the nitrogen excretion, then chromatography of urine containing equal amounts of nitrogen should result in equal amounts of total amino acid on the chromatogram.

A 19-month-old boy with deep second degree burns of 17% of his body surface (Chart 2 - Case 52-88) showed a pattern of amino acid excretion only

CASE NO.		WEAK							
52-88		STRONG							
ESSENTIAL AMINO ACIDS	MOLEC. WEIGHT	DAYS POST BURN							
		1	2	3	4	5	6	7	8
THREONINE	105	WEAK		STRONG					
VALINE	117								
LEUCINE	131		STRONG						
ISOLEUCINE	131								
LYSINE	146								
METHIONINE	149	WEAK							
PHENYLALANINE	165								
TRYPTOPHAN	204								
TOTAL		2	1	1					
NON-ESSENTIAL AMINO ACIDS									
GLYCINE	75	WEAK	STRONG	STRONG	STRONG				
ALANINE	89		STRONG	STRONG	STRONG				
SERINE	105		STRONG	STRONG	STRONG				
PROLINE	115								
HYDROXYPROLINE	131								
ASPARTIC ACID	133								
HISTIDINE	155	WEAK		STRONG	STRONG				
ARGININE	174	WEAK		STRONG	STRONG	WEAK			
TYROSINE	181								
GLUTAMIC ACID	183		WEAK	WEAK	WEAK	STRONG			
CYSTINE	240								
TOTAL		5	5	5	5	3			
UNKNOWN									
TAURINE									
GLUTAMINE		STRONG	WEAK	STRONG	STRONG				
TOTAL		8	8	8	6	5			

CHART 2

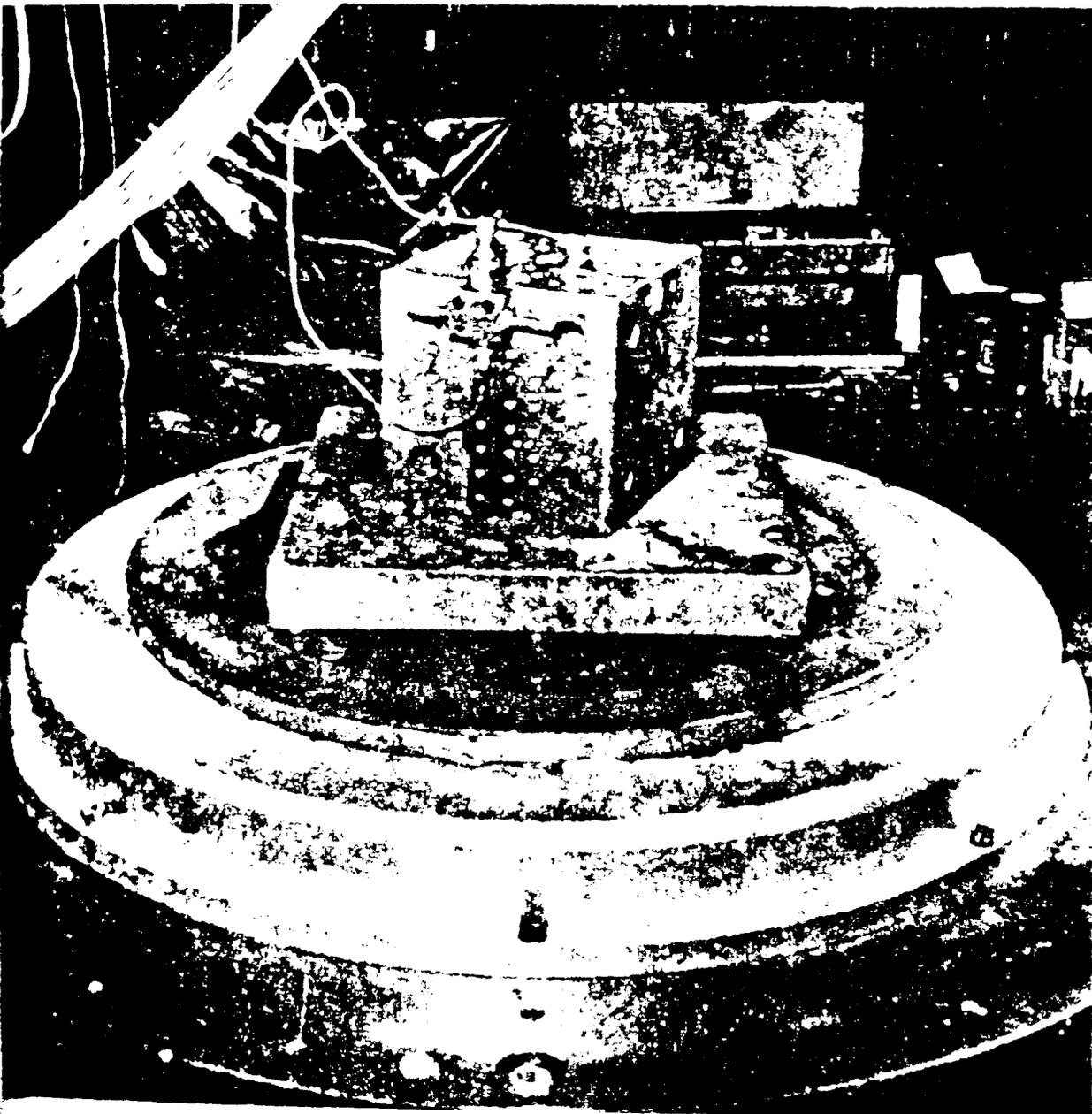
slightly greater than normal. If taurine and glutamine were to be excluded, the pattern might be considered at the upper limit of our "normals."

A similar pattern is seen in the amino acid excretion of a 42-year-old man who suffered a thermal burn of 30% of his body surface; about half of this was full thickness (Chart 3, Case 52-70). Amino-aciduria subsided somewhat after the first four weeks.

Chart 4 (Case 52-51) demonstrates the amino acids present in the urine of a 30-year-old lineman who suffered deep, high voltage, electrical burns of the entire right arm and both thighs. The arm was amputated at the shoulder and both legs were radically debrided at the time of admission, 48 hours after injury. His convalescence was uneventful. Taurine and glutamine were again present. The over-all amino acid excretion appeared greater than normal throughout the period of observation. In chart 4A this patient's nitrogen balance has been charted synchronously with the number of amino acids excreted. Nitrogen balance was calculated by subtracting the grams of nitrogen (Kjeldahl) in the urine, feces, and dressings from grams of nitrogen administered by mouth and parenterally. It is of interest to note the relative constancy of the amino-aciduria despite the shifts in nitrogen balance.

Chart 5 (Case 52-90) represents the amino-aciduria of a 23-year-old man who suffered thermal burns of 40% of his total body surface, most of which were full thickness. Amino-aciduria was still present after forty days, at which time many areas were still unhealed. After 139 days, when grafting had been completed, reduction in amino acid excretion, particularly as regards the "essential" amino acids, is evident.

The amino acid pattern of a 45-year-old woman with 50% body surface burns, over half of which were full thickness, is summarized in Chart 6 (Case 52-92). She died seven days after burning, while in oliguria. The serum nonprotein nitrogen had risen from a value of 31 mg. percent at the time of admission to



PHOTOGRAPH 1

TYPICAL TEST SETUP, Z AXIS

CASE NO.		WEAK	AD = Admission Spec.									
52-51		STRONG	0 = Spec. remainder of admission day.									
ESSENTIAL AMINO ACIDS	MOLEC. WEIGHT	AD.	0	1	2	3	4	5	6	7	8	
THREONINE	105	■									≡	
VALINE	117	■										
LEUCINE	131											
ISOLEUCINE	131											
LYSINE	146			≡	■			■	≡			
METHIONINE	149											
PHENYLALANINE	165											
TRYPTOPHAN	204											
TOTAL			1		2	2	1	1	2	2	2	1
NON-ESSENTIAL AMINO ACIDS												
GLYCINE	75	■										
ALANINE	89	■										
SERINE	105	■										
PROLINE	115	■										
HYDROXYPROLINE	131											
ASPARTIC ACID	133											
HISTIDINE	155	■			■		≡	■	≡	≡		
ARGININE	174											
TYROSINE	181											
GLUTAMIC ACID	183											
CYSTINE	240											
TOTAL			4	3	4	4	3	4	4	4	4	3
UNKNOWN												
TAURINE		■										
GLUTAMINE				≡					≡			
TOTAL			7	4	8	8	5	6	8	8	7	5

CHART 4

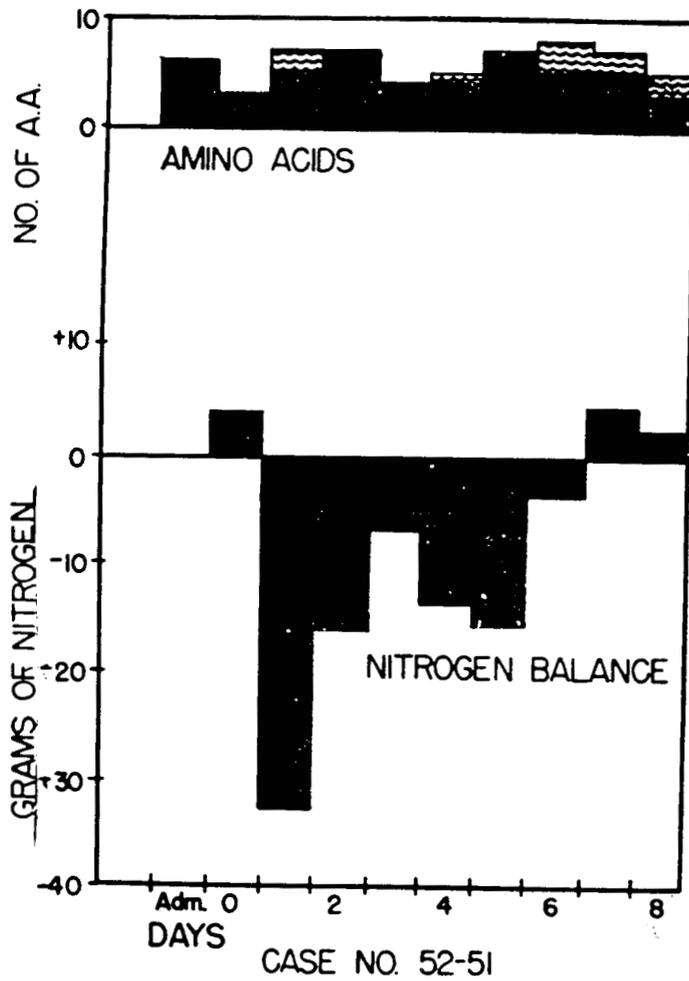


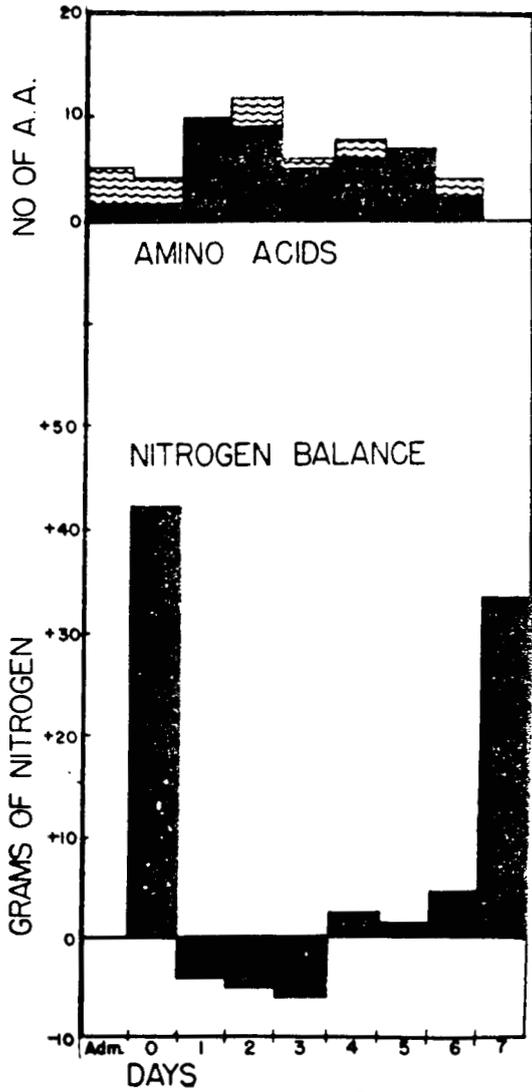
CHART 4a

CASE NO.		WEAK		AD. Admission Spec.												
52-90		STRONG														
ESSENTIAL AMINO ACIDS	MOLEC. WEIGHT	DAYS POST BURN														
		AD	1	2	3	4	5	6	7	8	9	10	11	40-41	42	139-40-41
THREONINE	105															
VALINE	117															
LEUCINE	131															
ISOLEUCINE	131															
LYSINE	146															
METHIONINE	149															
PHENYLALANINE	165															
TRYPTOPHAN	204															
TOTAL				5	4	2	3	5	3	3	4	4	4	4	4	
NON-ESSENTIAL AMINO ACIDS																
GLYCINE	75															
ALANINE	89															
SERINE	105															
PROLINE	115															
HYDROXYPROLINE	131															
ASPARTIC ACID	133															
HISTIDINE	155															
ARGININE	174															
TYROSINE	181															
GLUTAMIC ACID	183															
CYSTINE	240															
TOTAL		1	4	5	4	5	5	5	5	4	5	4	4	5	5	5
UNKNOWN																
TAURINE																
GLUTAMINE																
TOTAL		3	6	12	9	8	10	12	10	9	11	10	10	11	11	11

CHART 5

CASE NO.		WEAK	AD = Admission Spec.									
52-92		STRONG	0 = Spec. remainder of admission day									
ESSENTIAL AMINO ACIDS	MOLEC. WEIGHT	AD	0	1	2	3	4	5	6	7	8	9
THREONINE	105	WEAK										
VALINE	117	STRONG										
LEUCINE	131	WEAK										
ISOLEUCINE	131											
LYSINE	146											
METHIONINE	149											
PHENYLALANINE	165											
TRYPTOPHAN	204											
TOTAL			1	2	4	4	2	3	1			
NON-ESSENTIAL AMINO ACIDS												
GLYCINE	75	WEAK										
ALANINE	89	STRONG										
SERINE	105											
PROLINE	115											
HYDROXYPROLINE	131											
ASPARTIC ACID	133											
HISTIDINE	155											
ARGININE	174											
TYROSINE	181											
GLUTAMIC ACID	183	WEAK										
CYSTINE	240											
TOTAL			3	2	4	5	3	4	5	3		
UNKNOWN												
TAURINE												
GLUTAMINE												
TOTAL			5	4	10	12	6	8	7	4		

CHART 6



CASE NO. 52-92

CHART 6 a

135 mg. percent two days before death. Chart 6A demonstrates that the period of amino-aciduria corresponded roughly to the period of greatest nitrogen negativity. However, amino-aciduria persisted after restoration of positive nitrogen balance, suggesting that the quantitative and qualitative excretion of amino acids was independent of the over-all nitrogen balance.

A 64-year-old woman with a thermal burn of 64% of her body surface, more than half of which was full thickness, died on her tenth hospital day. Amino-aciduria persisted, though subsiding, until the time of death (Chart 7, Case 52-83). In Chart 7A the amino acid excretion again fluctuates independently of over-all nitrogen balance. The terminal reduction of amino acid excretion may have been due to a reduced glomerular filtration.

The Unburned

Chromatography of the preoperative urine specimens of the five patients undergoing surgery revealed a normal pattern in every case. Minor procedures such as hemorrhoidectomy and excision of fissure in ano resulted in little if any deviation from normal. Partial gastrectomy (Chart 8, Case S-3) resulted in somewhat less amino-aciduria than that seen in Chart 5. Hysterectomy and herniorrhaphy resulted in a pattern which lay between that of gastrectomy and hemorrhoidectomy.

The three patients with Cushing's disease showed a greater than normal urinary excretion of amino acids before operation. Chart 9 illustrates the typical findings in one of these cases. The patient was a 42-year-old woman with a ruddy face, abnormal fat distribution, hirsutism, and hypertension. A two-stage, radical, subtotal, adrenalectomy was carried out under cyclopropane anesthesia. The postoperative course was uneventful and the dosage of cortisone was gradually reduced from 200 mg. per day to a daily maintenance dose of 30 mg. over a period of two weeks. After this maintenance

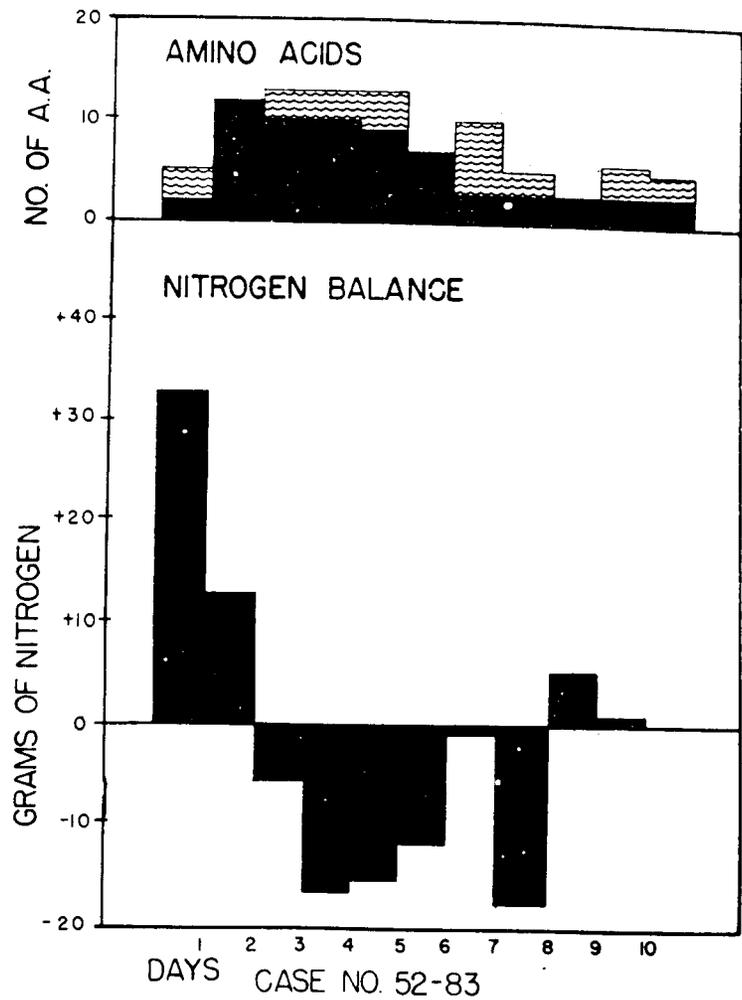


CHART 7a

ESSENTIAL AMINO ACIDS	AMINO MOLEC. WEIGHT	DAYS																		
		PRE	0	1	2	3	4	6	8	9	10									
THREONINE	105			WEAK																
VALINE	117			WEAK																
LEUCINE	131			WEAK																
ISOLEUCINE	131																			
LYSINE	146																			
METHIONINE	149																			
PHENYLALANINE	165																			
TRYPTOPHAN	204																			
TOTAL					2	2	2		1	3						1				
NON-ESSENTIAL AMINO ACIDS																				
GLYCINE	75																			
ALANINE	89																			
SERINE	105																			
PROLINE	115																			
HYDROXYPROLINE	131																			
ASPARTIC ACID	133																			
HISTIDINE	155																			
ARGININE	174																			
TYROSINE	181																			
GLUTAMIC ACID	183																			
CYSTINE	240																			
TOTAL			3	5	4	5	3	4	6	2	2	3								
UNKNOWN					1	2	1	2	1	1	1	1								
TAURINE																				
GLUTAMINE																				
TOTAL			5	8	9	10	4	8	12	4	3	6								

CHART 8

CASE NO. 41		WEAK		STRONG		PRE-OP							POST-OP						
ESSENTIAL AMINO ACIDS	MOLEC. WEIGHT	1	2	3	4	5	6	7	1	2	3	4	5	6	7	8			
THREONINE	105	■																	
VALINE	117																		
LEUCINE	131																		
ISOLEUCINE	131																		
LYSINE	146					■													
METHIONINE	149																		
PHENYLALANINE	165																		
TRYPTOPHAN	204																		
TOTAL		1	1			1													
NON-ESSENTIAL AMINO ACIDS																			
GLYCINE	75	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
ALANINE	89	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
SERINE	105	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
PROLINE	115																		
HYDROXYPROLINE	131																		
ASPARTIC ACID	133	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
HISTIDINE	155	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
ARGININE	174																		
TYROSINE	181																		
GLUTAMIC ACID	183																		
CYSTINE	240																		
TOTAL		3	5	4	1	4	2	3	2	2	1	2	3	3	3	3			
UNKNOWN																			
TAURINE																			
GLUTAMINE																			
TOTAL		5	7	5	2	5	3	4	3	2	1	3	3	4	3	4			

CHART 9

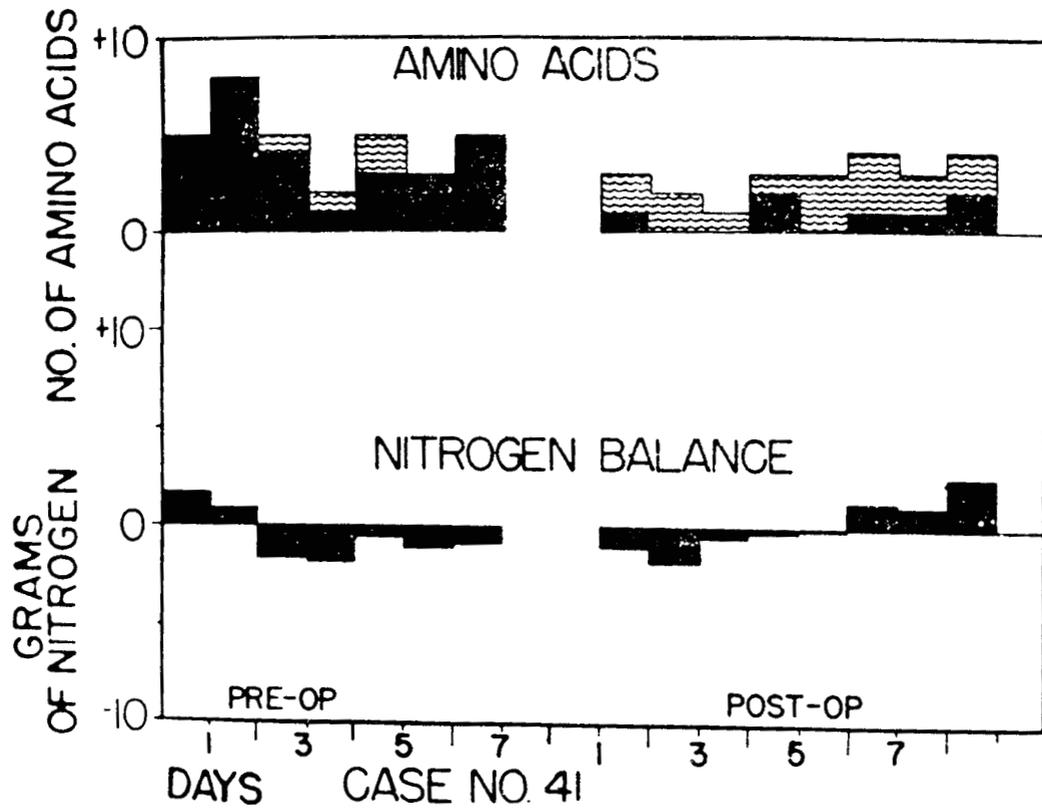


CHART 9a

dose had been established, daily urinary collections were carried out for five days. Despite the possible residual effects of surgery, the minimal amino acid excretion is striking. Chart 9 A shows the reduction of amino-aciduria in the postoperative phase as well as the over-all nitrogen metabolism.

Discussion

Amino-aciduria accompanied the increased urinary nitrogen loss which follows severe thermal trauma. It was also present after major surgical procedures. It consisted of an increase in both the lower molecular weight, "nonessential" amino acids normally found in urine and the higher molecular weight, "essential" amino acids not usually present. There was some suggestion that both the qualitative and quantitative aspects of the amino-aciduria may be proportional to the severity of the trauma. Patients who were not burned severely enough to endanger their lives and patients undergoing minor surgical procedures showed only minimal amino acid excretion with a preponderance of "nonessential" amino acids. Those dying of their burns or undergoing major surgery had a massive excretion of both "essential" and "nonessential" amino acids.

The actual mechanism of this amino-aciduria is not clear. There are three possibilities. Amino-aciduria may result when elevated blood amino acid levels exceed the renal threshold. With normal blood levels, renal damage and failure of tubular reabsorption may cause amino-aciduria. Finally, amino-aciduria may be seen with normal blood levels and "intact" kidneys in certain "metabolic" disorders -- i.e., "primary amino-aciduria" as in the de Toni-Fanconi syndrome and cystinuria. Although the available data are inadequate to rule out such a mechanism in thermal trauma, a metabolic disorder of this sort seems unlikely. It would seem most likely, then, that the escape of amino acids observed is due to either elevated plasma amino

acid levels or renal dysfunction (although no evidence for the latter has been found), or a combination of these two mechanisms.

Levenson et al¹⁴ have demonstrated elevation of alpha amino nitrogen in the blood of eight of twelve patients with thermal burns in the first 24 hours after injury. However, marked hemoconcentration was present in these patients. Repeated plasma amino nitrogen levels determined in our patients when hemoconcentration was not present were always found to be within normal limits, i.e., less than 5 mg. percent.

Everson and Fritschel¹⁵ failed to show any rise in plasma amino nitrogen subsequent to anesthesia and surgery. Friedberg and Greenberg¹⁶ and Li et al¹⁷ suggest that cortical extract or adrenocorticotrophic hormone may increase the level of plasma amino acids. Grief,¹⁸ however, found a significant fall in the plasma amino acid level of a hypopituitary patient receiving adrenocorticotrophic hormone. Russell¹⁹ found that the increase in blood urea in nephrectomized rats resulting from intravenously administered amino acids was markedly reduced by this pituitary hormone.

The high protein intake administered to convalescing burned patients might be a factor in elevating the plasma amino acid level. Dent and Schilling²⁰ showed that after ingestion of proteins large rises occurred in the concentration of many amino acids in the portal blood of dogs, which could be accompanied by amino-aciduria. It is doubtful that a high protein intake played a significant role in our patients, since the amino-aciduria was subsiding as the patients improved clinically and were able to ingest more protein. In addition, the surgical patients received little or no protein during the first days of convalescence.

Certainly all these factors, protein breakdown, shock, and increased steroid production, are operating in the burned and traumatized patients. However, it seems unlikely that increased blood concentration of amino acids alone can account for the observed amino-aciduria.

After administration of ACTH to a patient, Bonzoni et al²¹ found an increase in urinary amino acid excretion, which returned to normal upon cessation of therapy. However, Russell and Wilhelm²² demonstrated an improved rate of de-amination in adrenalectomized rats when cortisone was administered. Burnett²³ suggested that steroid administration improved tubular reabsorption in Addison's disease. Cogan et al²⁴ felt that cortisone caused increased tubular reabsorption of intravenously administered amino acids in treated Addisonians. It is possible that one of the adrenal steroids other than cortisone is responsible for failure of tubular reabsorption, but we have no evidence for this at present. Since adrenal hyperfunction is probably present in all our burns, cortisone may be suspected as a causative factor.

The kidney may play a specific role in the amino-aciduria of trauma. Amino acids seem to resemble glucose in their renal behavior. However, the mechanism of tubular reabsorption must differ since phlorizin poisoning does not produce amino-aciduria. Since amino acids filter freely through the glomerulus, amino-aciduria may result from failure of tubular reabsorption. Pitts²⁵ has shown a competitive tubular reabsorption for creatin and glycine. Since numerous investigators^{26,27,28} have shown the presence of creatinuria in burns, a possible explanation for an accompanying amino-aciduria is suggested. However, since such competition exists only at very high plasma levels, this factor is probably of no importance.

Finally, the influence of amino acids themselves on the renal tubules must be considered. Kamin and Handler²⁹ have shown that the excretion of threonine and histidine was easily effected by the presence of an unusual quantity of any other amino acid in the plasma. Perhaps the presence of a disorderly, albeit not elevated, pattern of amino acids in the renal tubule is enough to initiate a disturbance of tubular reabsorption.

Summary

An abnormal amino-aciduria consisting of excretion of "essential" amino acids not normally present in urine as well as increased quantities of "nonessential" amino acids was found in the urines of severely burned or traumatized patients.

The qualitative and quantitative aspects of this amino-aciduria may be proportional to the severity of the trauma. The amino acid excretion was influenced by but not completely dependent upon the total urinary nitrogen. Hyperactivity of the adrenal cortex as seen in Cushing's disease resulted in a moderate degree of amino-aciduria. The latter effect was not reproduced by a daily maintenance dose of 30 mg. cortisone after bilateral subtotal adrenalectomy.

This escape of amino acids is most likely due to a failure of tubular reabsorption. The reason for the latter is not clear but may be due to some adrenal steroid other than cortisone, to an abnormal amino acid pattern presented to the tubule, or to some other factor.

Acknowledgment

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URINARY LOSS OF AMINO ACIDS AFTER SURGERY

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Introduction

It is well known that patients who have undergone surgery lose large amounts of nitrogen in their urines in the immediate postoperative period.^{1,2} However, it is not known in what proportions these nitrogen containing constituents are excreted during this negative nitrogen phase. Since urine normally contains only traces of free amino acids amounting to one or two percent of the total urinary nitrogen,³ it was thought of interest to study the amino acids present in the urines of patients undergoing surgery of varying degrees of magnitude.

Methods

Twenty-four hour urine collections were obtained from patients on the surgical wards of the Massachusetts General Hospital; aliquots were analyzed for nitrogen (Kjeldahl) and volumes containing equal amounts of nitrogen were chromatographed two dimensionally on paper using the technic of Dent.⁴ Phenol and water was used as the first solvent and butanol-propionic acid and water as the second.⁵ The amino acids were developed by spraying with 0.1% ninhydrin in ethanol. Using this technic, urine of normal people contains mainly glycine and alanine and occasional small quantities of serine, glutamic acid, and threonine.^{6,7,8} In general, only the lower molecular weight, nonessential amino acids are to be found in urine. Chart 1 represents a typical daily normal excretion pattern of one of our laboratory personnel.

Five patients were studied. The operations represented are: excision of anal fissure, hemorrhoidectomy, inguinal herniorrhaphy, total hysterectomy and perineorrhaphy, and subtotal gastrectomy.

ESSENTIAL AMINO ACIDS	MOLEC. WEIGHT	DAYS				
		1	2	3	4	5
THREONINE	105					WEAK
VALINE	117					
LEUCINE	131					
ISOLEUCINE	131					
LYSINE	146		STRONG			
METHIONINE	149					
PHENYLALANINE	165					
TRYPTOPHAN	204					
TOTAL			1			1
NON-ESSENTIAL AMINO ACIDS						
GLYCINE	75	WEAK	STRONG	WEAK		
ALANINE	89		STRONG	WEAK		
SERINE	105	WEAK	WEAK	WEAK		
PROLINE	115					
HYDROXYPROLINE	131					
ASPARTIC ACID	133					
HISTIDINE	155			WEAK		
ARGININE	174					
TYROSINE	181					
GLUTAMIC ACID	183					
CYSTINE	240					
TOTAL		2	2	4	2	3
UNKNOWN						
TAURINE						
GLUTAMINE						
TOTAL		2	3	4	2	4

CHART I

Results

Chart 2 represents the effects of the minimal anesthesia and surgery required for excision of a small anal fissure. As can be seen, there is little deviation from normal.

Hemorrhoidectomy, a procedure of somewhat greater magnitude, produced a slight but definite increase in amino acid excretion. (Chart 3)

Charts 4 and 5 represent the amino-aciduria occurring after herniorrhaphy and hysterectomy respectively. The greater loss of amino acids as compared to the two previous, lesser procedures is obvious.

The marked amino-aciduria produced in a patient undergoing subtotal gastrectomy is illustrated by Chart 6.

Amino-aciduria accompanies the increase in urinary nitrogen loss which follows surgical trauma. This amino-aciduria consists of an increase in both the lower molecular weight nonessential amino acids normally found in urine as well as of the higher molecular weight essential amino acids not usually present. The degree of amino-aciduria seems proportional to the magnitude of the surgical trauma.

Discussion

There are three causes of amino-aciduria.⁷ It may result if blood amino acid levels exceed the renal threshold. Another cause with normal amino acid blood levels is renal damage or failure of tubular reabsorption. A third mechanism is the amino-aciduria occurring with normal blood levels of amino acids and normal renal function -- the primary amino-aciduria of various metabolic diseases. The latter need not concern us.

Everson and Fritschel⁹ have shown no rise in plasma amino nitrogen subsequent to anesthesia and surgery. Man¹⁰ has found a postoperative fall in these levels. It would seem then that the amino-aciduria seen after surgery

ESSENTIAL AMINO ACIDS	MOLEC. WEIGHT	DAYS			
		Pre	0	1	2
THREONINE	105				
VALINE	117				
LEUCINE	131				
ISOLEUCINE	131				
LYSINE	146				
METHIONINE	149				
PHENYLALANINE	165				
TRYPTOPHAN	204				
TOTAL					
NON-ESSENTIAL AMINO ACIDS					
GLYCINE	75				
ALANINE	89	WEAK	STRONG	WEAK	
SERINE	105	WEAK	STRONG		
PROLINE	115				
HYDROXYPROLINE	131				
ASPARTIC ACID	133				
HISTIDINE	155				
ARGININE	174				
TYROSINE	181				
GLUTAMIC ACID	183				
CYSTINE	240				
TOTAL		3	3	2	2
UNKNOWN		1			
TAURINE					
GLUTAMINE		WEAK			
TOTAL		5	3	2	2

CHART 2

ESSENTIAL AMINO ACIDS	MOLEC. WEIGHT	DAYS					
		Pre	0	1	2	3	4
THREONINE	105		WEAK				
VALINE	117						
LEUCINE	131						
ISOLEUCINE	131						
LYSINE	146						STRONG
METHIONINE	149						
PHENYLALANINE	165						
TRYPTOPHAN	204						
TOTAL			1			1	
NON-ESSENTIAL AMINO ACIDS							
GLYCINE	75		STRONG				STRONG
ALANINE	89		WEAK				WEAK
SERINE	105				WEAK		
PROLINE	115						
HYDROXYPROLINE	131						
ASPARTIC ACID	133		WEAK				
HISTIDINE	155						
ARGININE	174						
TYROSINE	181						
GLUTAMIC ACID	183						
CYSTINE	240						
TOTAL			2	3	1	3	2
UNKNOWN							
TAURINE							STRONG
GLUTAMINE			STRONG		STRONG		
TOTAL			2	5	1	4	4

CHART 3

ESSENTIAL AMINO ACIDS	MOLEC. WEIGHT	DAYS										
		0	1	2	3	4	5	6	7	8		
THREONINE	105				WEAK							
VALINE	117											
LEUCINE	131											
ISOLEUCINE	131											
LYSINE	146											
METHIONINE	149											
PHENYLALANINE	165											
TRYPTOPHAN	204											
TOTAL												
NON-ESSENTIAL AMINO ACIDS												
GLYCINE	75											
ALANINE	89				WEAK							
SERINE	105											
PROLINE	115											
HYDROXYPROLINE	131											
ASPARTIC ACID	133											
HISTIDINE	155											
ARGININE	174											
TYROSINE	181											
GLUTAMIC ACID	183											
CYSTINE	240											
TOTAL												
UNKNOWN			3	4	3		1	4	3	3		
TAURINE												
GLUTAMINE												
TOTAL			4	7	4		2	6	6	5		

CHART 4

ESSENTIAL AMINO ACIDS	MOLEC. WEIGHT	DAYS				
		0	1	2	3	4 5
THREONINE	105					
VALINE	117					■
LEUCINE	131					
ISOLEUCINE	131					
LYSINE	146					
METHIONINE	149					
PHENYLALANINE	165					
TRYPTOPHAN	204					
TOTAL						
NON-ESSENTIAL AMINO ACIDS						
GLYCINE	75					■
ALANINE	89					■
SERINE	105					■
PROLINE	115					
HYDROXYPROLINE	131					
ASPARTIC ACID	133					■
HISTIDINE	155					
ARGININE	174					
TYROSINE	181					
GLUTAMIC ACID	183					
CYSTINE	240					
TOTAL			4	4	4	4
UNKNOWN						
TAURINE						■
GLUTAMINE						■
TOTAL			6	7	7	8

CHART 5

ESSENTIAL AMINO ACIDS	MOLEC. WEIGHT	DAYS																		
		PRE	0	1	2	3	4	6	8	9	10									
THREONINE	105				STRONG															
VALINE	117																			
LEUCINE	131																			
ISOLEUCINE	131																			
LYSINE	146																			
METHIONINE	149																			
PHENYLALANINE	165																			
TRYPTOPHAN	204																			
TOTAL				2	2	2		1	3											
NON-ESSENTIAL AMINO ACIDS																				
GLYCINE	75																			
ALANINE	89																			
SERINE	105																			
PROLINE	115																			
HYDROXYPROLINE	131																			
ASPARTIC ACID	133																			
HISTIDINE	155																			
ARGININE	174																			
TYROSINE	181																			
GLUTAMIC ACID	183																			
CYSTINE	240																			
TOTAL		3	5	4	5	3	4	6	2	2	3									
UNKNOWN																				
TAURINE																				
GLUTAMINE																				
TOTAL		5	8	9	10	4	8	12	4	3	6									

CHART 6

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PAPER CHROMATOGRAPHY OF FREE AMINO ACIDS IN BLOOD PLASMA*

by

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Paper chromatography of the free amino acids present in blood has presented numerous technical difficulties which have resulted in failure to utilize this technique. Paper partition chromatography has found increasingly widespread use in the chemical analysis of urine and tissue extracts. Blood plasma or serum, however, contains three substances which make the application of identical techniques impossible. These are salts, proteins, and lipid materials. An electrolytic desalting machine has been devised and utilized for removal of the salts.^{1,2,3} However, it has been shown that there is a loss of some of the amino acids during the process of desalting.^{4,5} In addition, the method is cumbersome. The use of the usual protein-precipitating agents often results in a loss of numerous amino acids.⁶ Deproteinization can be effected by ultrafiltration, but this too is tedious. The presence of lipid material results in a technically unsatisfactory chromatogram with smearing and smudging.

The most efficient way to achieve separation of the amino acids in blood would be by a series of ion-exchange resins. This procedure is best illustrated by the recent work of Stein and Moore.⁷ However, this technique is extremely tedious and time consuming and does not lend itself to the study of a large number of samples of small volume and concentration.

Recent reports in the French literature⁸ have suggested a chemical method of preparing blood for paper chromatography. We have modified such a method and have been able to produce satisfactory chromatograms of human blood plasma or serum. We have summarized our findings in the form of a "map" of the free amino acid and ninhydrin-reacting compounds which are to be found in normal human blood by our technique.

Methods

One cc. of blood plasma is placed in a crystallizing dish measuring 50 by 35 mm. The latter is placed in a vacuum desiccator containing technical grade sulphuric acid and the plasma is allowed to dry overnight.

To the dry plasma 8 cc. of acetone containing 1.6 cc. of 6 N HCl per 100 cc. is added. The dried plasma is then ground very finely and the crystallizing dish covered with a 3 x 3 in. glass plate smeared with stopcock grease. The dish is then agitated for a period of two hours. The acetone solution is then transferred to a 15 cc. centrifuge tube, as much of the precipitated protein being transferred as possible. To the residue in the crystallizing dish is then added 5 cc. of the acetone and HCl mixture. The agitation is then repeated. The transferred solution in the centrifuge tube is centrifuged for ten minutes. The supernatant is transferred to a test tube. The second batch of the acetone solution in the crystallizing dish is transferred to the original centrifuge tube, including again as much precipitate as possible. The second batch is then centrifuged for ten minutes and the supernatant removed and added to the first supernatant. The procedure is repeated a third time so that at the end of the procedure the precipitated proteins are washed three times with the acetone-HCl mixture and the washing is collected in a single test tube.

A special test tube 16 mm. in diameter and 50 mm. in height is placed in a water bath of 37° C. A stream of air dried by passing through a tube of calcium chloride is blown near the top of the tube by a small pipette. The tube is then filled with the acetone extracts of amino acid as evaporation occurs, and evaporation is carried out in successive fractions until the residue is left dry on the bottom of the small test tube. This residue will present a yellow-brown color. It contains the amino acids free of protein and salt but with lipids.

One-half cc. of distilled water is placed in the bottom of the test tube and the residue dissolved. An equal quantity of diethylether is then added and the tube agitated. The ether layer is then removed and this extraction is repeated twice more for a total of three extractions. This procedure removes the lipid materials.

The remaining aqueous solution is then placed once more in the vacuum desiccator over technical grade sulphuric acid and evaporated to dryness.

The dried residue is then dissolved in the desired volume of distilled water, usually 25 to 100 microliters, and applied to the filter paper for chromatography.

Chromatography can then be carried out in any of the usual methods. We prefer to use phenol and water as the first solvent and butanol-propionic acid and water as the second solvent. The dried two-dimensional chromatogram is sprayed with an ethanolic solution of 0.1% ninhydrin and the color allowed to develop overnight at room temperature.

Such preparation requires a period of two days. However, a large number of samples can be carried through simultaneously in a simple and uncomplicated fashion.

Results

The above-described analyses were carried out on the blood of numerous members of our laboratory personnel. Both plasma and serum were used, and in some cases pooled samples of plasma were utilized. The chromatograms obtained were quite similar in all cases. Figure 1 represents a composite "map" of the free amino acids and positive ninhydrin reacting compounds found in normal human blood by this technique.

In our experience, if the ether extraction is not carried out, the area of the chromatograph proximal to alanine is smudged on the chromatogram and the spots of this area lack sharpness.

Quantitative determination of the alpha amino nitrogen by the Van Slyke method at various stages of the procedure has shown that this technique results in a negligible loss of total alpha amino nitrogen.

Identification of the spots was made by addition of a pure amino acid to a sample before running it on the chromatogram and verifying the re-enforcement

in color of the particular spot and failure of a new spot to appear.

As can be seen from Figure 1, eleven ninhydrin-positive compounds have been identified and named on the chart. They are charted as solid black. Six additional spots have not been definitely identified and are therefore shaded and numbered one to six. Spot No. 1 lay in the tail from the cysteine, suggesting that it is a decomposition product of the latter. Other investigators have reported the presence of histidine, arginine, and lysine. They were not found in our material even after spraying the chromatograms with Pauly's diazo reagent. The failure to demonstrate these basic amino acids may be inherent in this method. Spots 2, 3, 4, and 5 remain unidentified. Spot No. 6 has a yellow color and appeared only in the pooled plasma. It never appeared in individual blood samples and therefore may not represent a normal constituent of human plasma. Further speculation as to the nature of these unidentified materials does not seem justified at present.

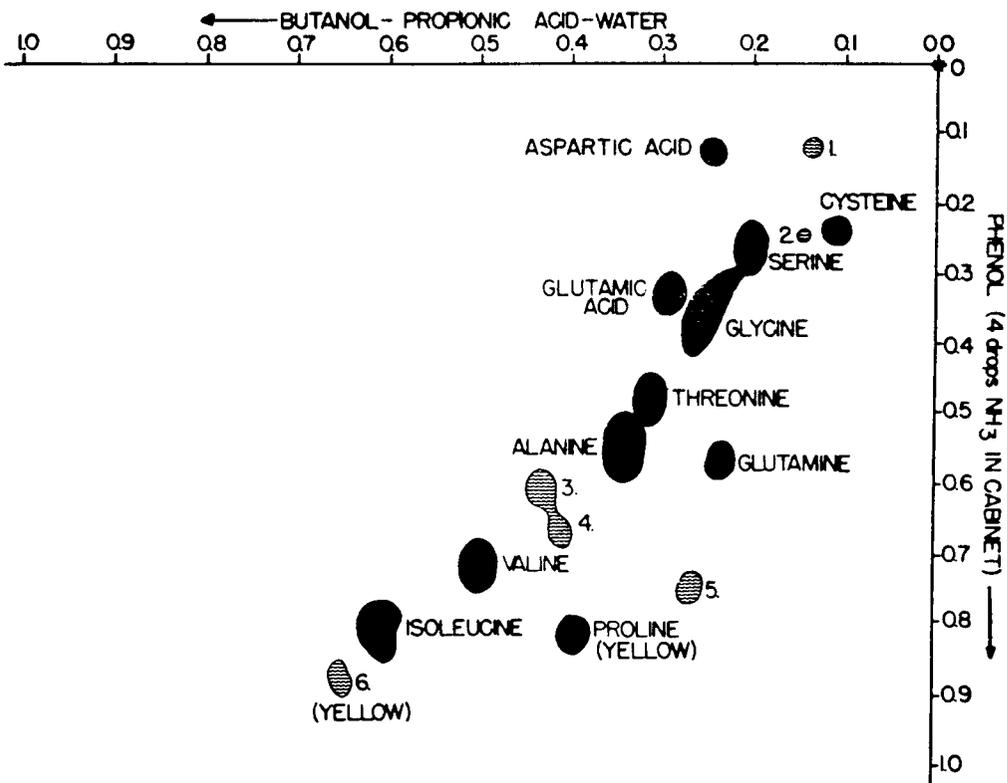
Summary

A method of preparing blood plasma or serum for paper chromatographic analysis has been described. The free alpha amino acids and ninhydrin-reacting substances found in normal human blood by this method have been described.

Acknowledgements

The authors wish to thank Drs. E. D. Churchill and Oliver Cope for providing laboratory facilities and encouragement.

POOLED HUMAN PLASMA



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LIVER BLOOD FLOW IN THERMAL TRAUMA

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The desirability of liver blood flow measurements was expressed at a recent meeting of the Subcommittee on Burns of the National Research Council.¹ At this meeting we stated that a method for such measurements was being developed in our laboratories. This report is a preliminary summary of our findings. The effect of thermal trauma on liver blood flow has not been previously reported.

Though the over-all shifts of body fluids and electrolytes in burns and shock states have been well described,² the dynamic effects of this redistribution on organ flow is poorly understood. The kidney is, perhaps, an exception, and the importance of maintaining adequate renal flow is obvious.

Another organ whose circulatory dynamics may be of equal import is the liver.^{3,4} Little is known of the effects of thermal trauma on liver circulation. One of the chief impediments in the way of a study of hepatic hemodynamics has been the lack of an adequate technique of quantitating liver blood flows. The Bromsulfalein⁵ method is complicated and not applicable to conditions where hemolysis may occur. A simple and reproducible method of measuring liver blood flow has recently been devised by Dobson and Jones.⁶

They have utilized the disappearance rate of a radioactive colloid, cleared from the blood by the reticulo-endothelial system. The colloid size is such that there is no extravascular leakage into the burn wound.

In actual practice four microcuries of radioactive colloidal chromic phosphate (P-32) is injected intravenously. Heparinized blood samples are withdrawn at 2, 3, 4, 6, 8, and 10-minute intervals, plated and assayed for radioactivity. The activity is plotted on semi-logarithmic paper as a function of time, and the best straight line fitted to the points. The slope of this line is the disappearance constant or the fraction of the blood volume flowing through the liver per minute. The normal adult male has been found to have approximately 24% of his blood volume circulating through his liver per minute.⁷

In order to determine the changes in liver blood flow occurring after thermal burns as well as to evaluate the effect of various accepted replacement therapies on liver blood flow, studies were carried out on experimental animals. Four dogs were subjected to a standardized severe 50% body surface burn by immersing them for twenty seconds to their xyphoid processes in water at 83° C. (Nembutal anesthesia).

Blood flow determinations were done before the burn and at 30-minute intervals after burning. Hematocrits were done at the time of each determination.

No treatment was administered the first dog. His preburn hematocrit was 47% and his liver flow was 69% of his blood volume/minute. (Dogs normally have much higher flows than human beings.)⁶ One-half hour after burning, his hematocrit had risen to 57% and his blood flow had fallen to 26%. After one hour the hematocrit was 61% and liver flow down to 10% -- a reduction in liver blood flow to 1/6 his normal value. (Chart 1) It is highly suggestive that the hemoconcentration and increased blood viscosity may play an important role in this reduction of liver blood flow. (The relation of hematocrit to viscosity has been established in this laboratory in relation to thermal trauma in dogs and the human being -- a reprint already submitted.⁸)

Since in an untreated burn (Chart 1) we know that reduction of circulating volume occurs and since the data of Chart 1 represents only the percentage of blood volume flowing through the liver per minute, it is obvious that the actual liver flow in terms of cc/minute must be reduced to alarmingly low values!

Since survival after severe thermal trauma may be equally dependent on adequate liver function as well as adequate renal function, the evaluation of the effectiveness of various blood replacements in maintaining liver hemodynamics would seem essential.

In a second dog subjected to an identical burn saline was administered intravenously at a standard rate² which is believed to be optimal in these circumstances. The obvious improvement in liver flow can be seen in Chart 2. One hour after burning, the hematocrit had risen to 56% but liver flow had been reduced only to 1/2 the pre-burn value.

Replacement with plasma (Chart 3) resulted in similar findings with reduction of liver flow to less than 1/2 the original value.

Two patients with extensive full thickness burns have been studied.

An 84-year-old man was admitted to the hospital with full thickness burns of 20% of his body surface. Flow determination was done shortly after his arrival when he had received but 500 cc. of saline and 500 cc. of plasma intravenously. As can be seen in Chart 4, his flow of 23% was normal despite a hematocrit of 53% at the time of the determination.

The second patient was a 45-year-old woman with full thickness burns of approximately 60% of her body surface. Daily liver flow determinations were done on three successive days. These revealed flows of 25, 23, and 20% with simultaneous hematocrits of 54, 45, and 46%, suggesting that the fluid therapy which had maintained normal hematocrits had provided for adequate liver blood flow.

It is planned to extend these studies to obtain simultaneous blood volumes by the radioactively- chromated (Cr 51) red cell method so that liver flows can be expressed in terms of cc/min. Such measurements should enable us to approach the ideal therapy of the burned patient. .

DOG #1 Untreated

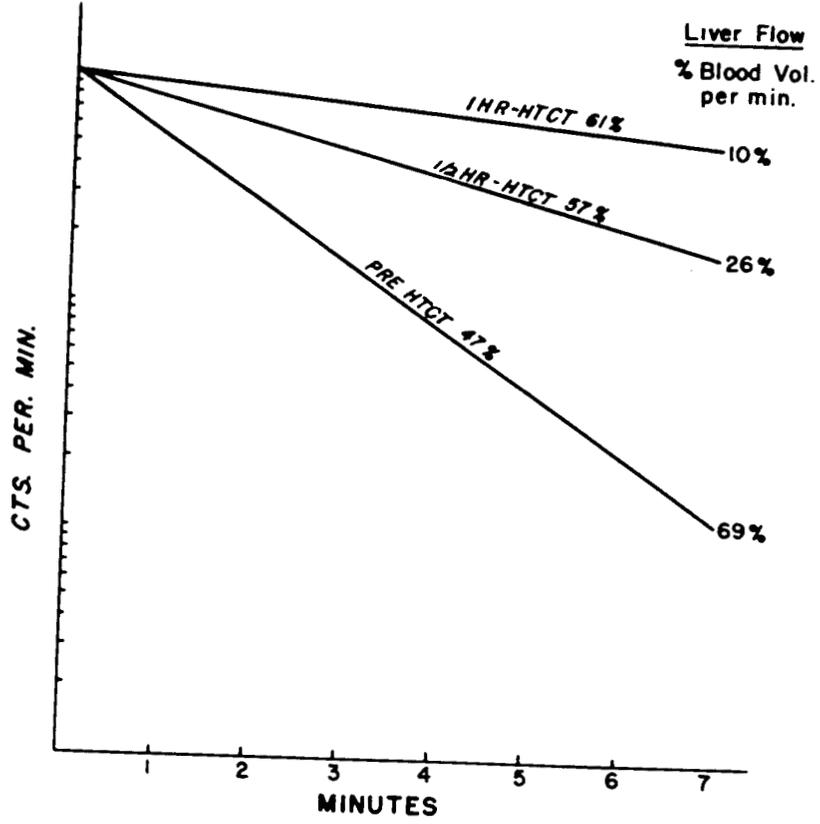


CHART 1

DOG 2# Saline Treatment

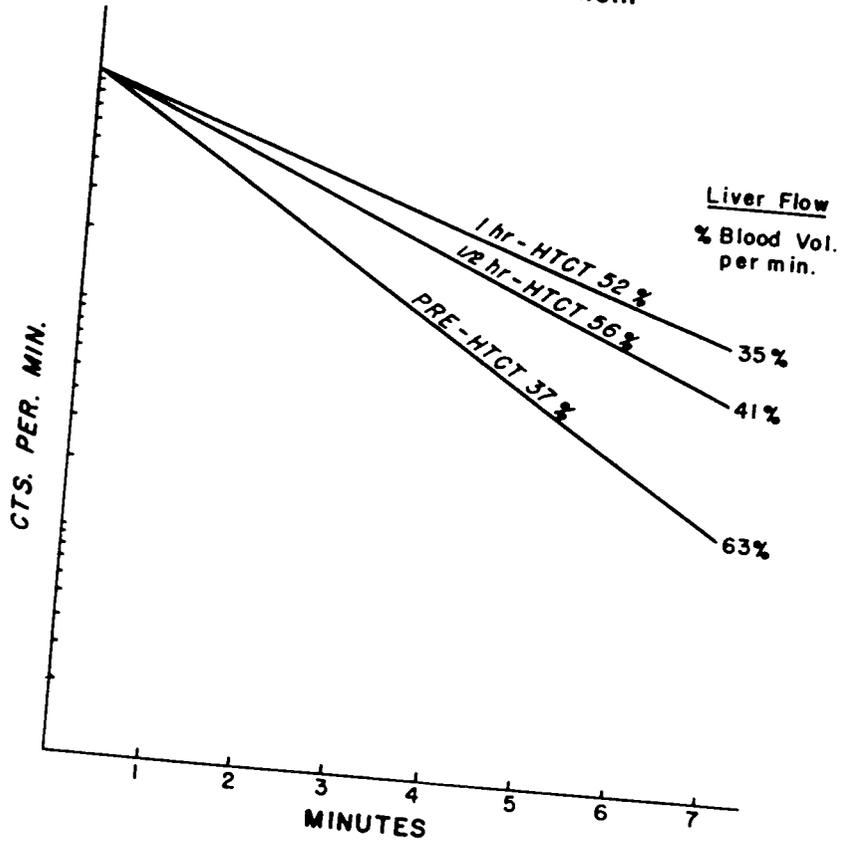


CHART 2

DOG #3 Plasma Treatment

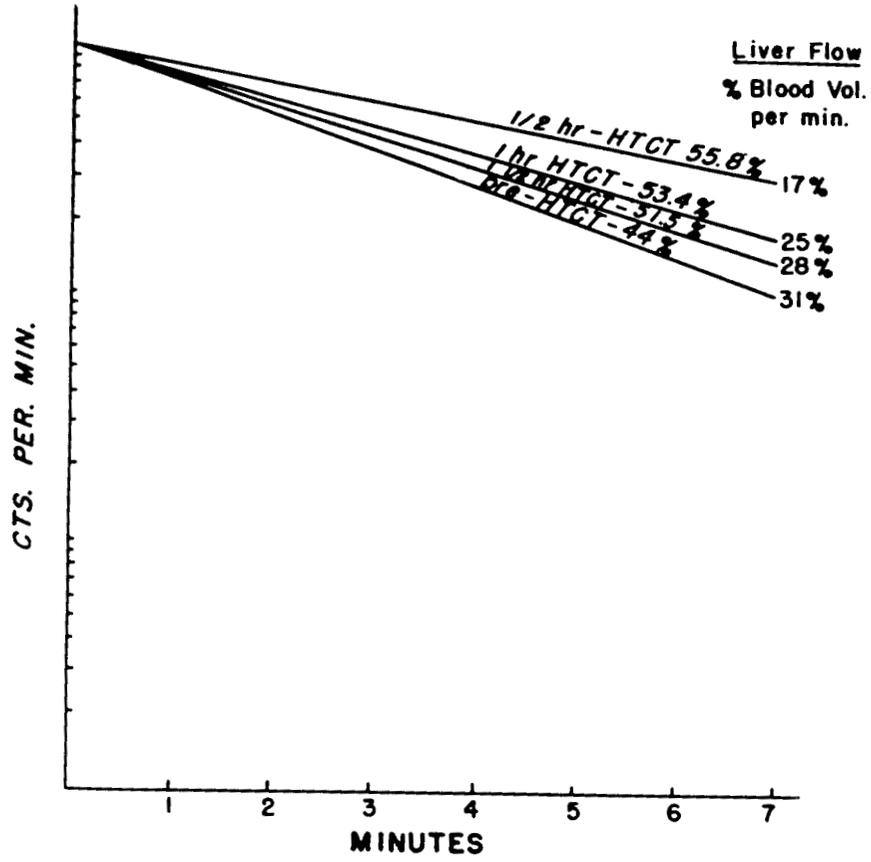


CHART 3

PATIENT I.

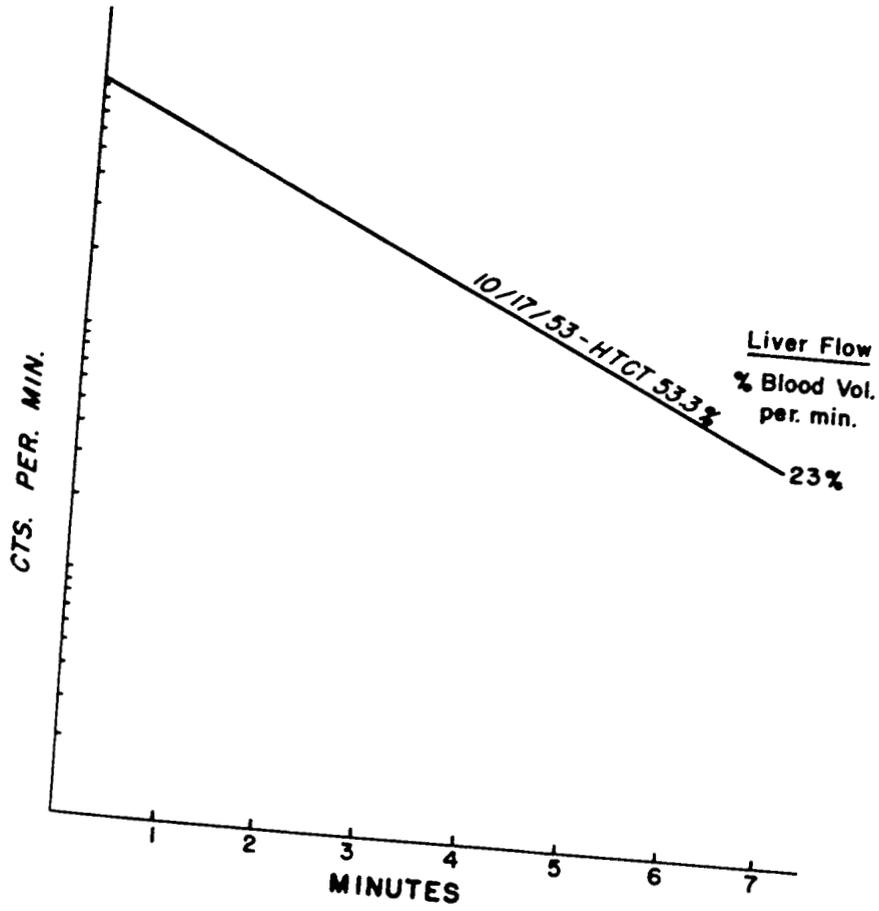


CHART 4

PATIENT 2.

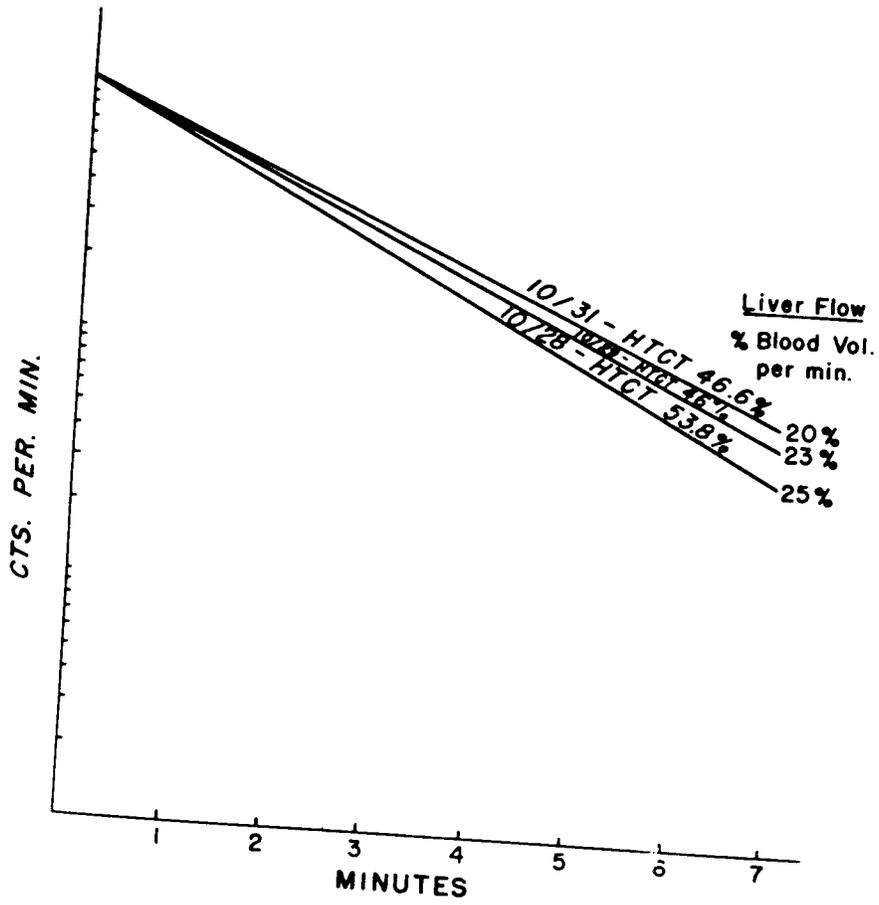


CHART 5

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POSTERIOR PITUITARY PROJECT

This project has been organized to evaluate the anti-diuretic mechanism in the burned patient. The mechanism involves the posterior pituitary gland and its anti-diuretic hormone (ADH) as well as the end organ, the renal tubule.

There have been two major stimuli to this study. The first consisted of observations of urine specific gravities over periods of one to eight weeks in 15 burned patients. In seven of these patients it ranged between 1.020 and 1.040, suggesting the possibility of a persistent anti-diuretic effect.

The second stimulus was the current knowledge of the physiology of the posterior pituitary. The major physiologic influences, that is changes in serum solute concentration and in "circulating blood volume", are disturbed in the severely burned patient. It seemed logical therefore to explore the competency of the anti-diuretic mechanism in such patients.

The problem of assessing anti-diuretic activity is central to this study. Bio-assay is time consuming and has not been uniformly reliable. The other available index of ADH activity is urine solute concentration. Renal water resorption and, hence, urine solute concentration is regulated exclusively by ADH according to the evidence now available. Furthermore this determination is rapid and reproducible to within ± 3 milliosmols per liter.

This study includes both animals and patients. The blood volume, thiocyanate space, and the serum solute level are correlated with the urine solute concentration in 24 hour specimens and with the pattern of urine solute concentrations following water loads. The concept of administering water loads is based on the work of Leaf who has demonstrated high urine solute levels and impaired water diuresis in the presence of a low serum solute concentration, probably because of the interference of volume regulating mechanisms.

Blood volume has been determined by the dilution of injected red blood cells tagged with radioactive chromium, the thiocyanate space by dilution of a known sample. Urine and serum solute concentrations are read on a sensitive freezing point apparatus.

Thus far four dogs and six burned patients have been studied.

The dogs have been followed for seven to nine days after a 50% body surface burn in water at 70°C. Of the first two dogs, one received no saline replacement, the other 200 cc normal saline on the day of the burn. Both were allowed to drink plain water.

The second pair was given about 60 meq sodium chloride per day in the form of N/3 saline in the drinking pans. They were offered no plain water.

Both pairs received approximately 8 ounces of meat daily.

Following the pre-burn determinations of thiocyanate space and Cr⁵¹ blood volume, the animals were burned as described. These determinations were repeated daily as nearly as possible thereafter. The animals were subjected to daily loads of 400 cc water by gastric tube and the diuretic response followed with serum and urine solute concentrations. Fluid intake and output as well as weight were recorded daily.

It was anticipated that the therapy described above might lead to a fall in serum solute concentration in one pair and to no change in the other, providing an informative contrast.

The normal serum solute concentration in dogs and human beings fluctuates in a narrow range about 290 milliosmols per liter. In the two dogs given little sodium chloride replacement the serum solute concentration fell to the range of 270 to 275 milliosmols per liter on the first and fourth days respectively and remained in that range.

The pair of dogs given ample sodium chloride replacement, on the other hand, maintained serum solute levels of over 300 milliosmols per liter

consistently after the burn.

The thiocyanate space expanded to approximately the same degree in both pairs of dogs and remained expanded throughout the period of observations between 110 and 125% of the original values.

Red blood cell volume fell (except in one dog in which the control determination was probably in error and in which determinations were omitted after the second post-burn day). The degree of this fall amounted to between 30 and 60% of the original red blood cell volume and was due primarily to the withdrawal of blood samples.

Blood volume remained near control values in each of the two low replacement dogs through the second and third post-burn days respectively when the last determinations were done. In one of the two dogs given high saline replacement the blood volume remained about 50% of the control value through the seventh post-burn day; in the other it was 125% of the pre-burn level on the eighth day.

Within the wide variations in thiocyanate spaces and blood volumes observed in these animals water diuresis was unimpaired (Chart II). In every case following the administration of 400 cc water through a gastric tube under conditions of rest a normal fall in urine solute concentration occurred.

The extremes of conditions under which this normal water diuresis occurred are as follows:

In dog A-3, a low replacement animal, a normal water diuresis was observed when the serum solute concentration was in the range of 271 to 270. Although no data are available for the spaces on this day, five previous determinations of the thiocyanate space had shown it to be expanded between 112 and 159% of the pre-burn value.

Dog B-1, a high replacement animal, displayed a normal water diuresis when the thiocyanate space was 111% and the blood volume 57% of pre-burn values.

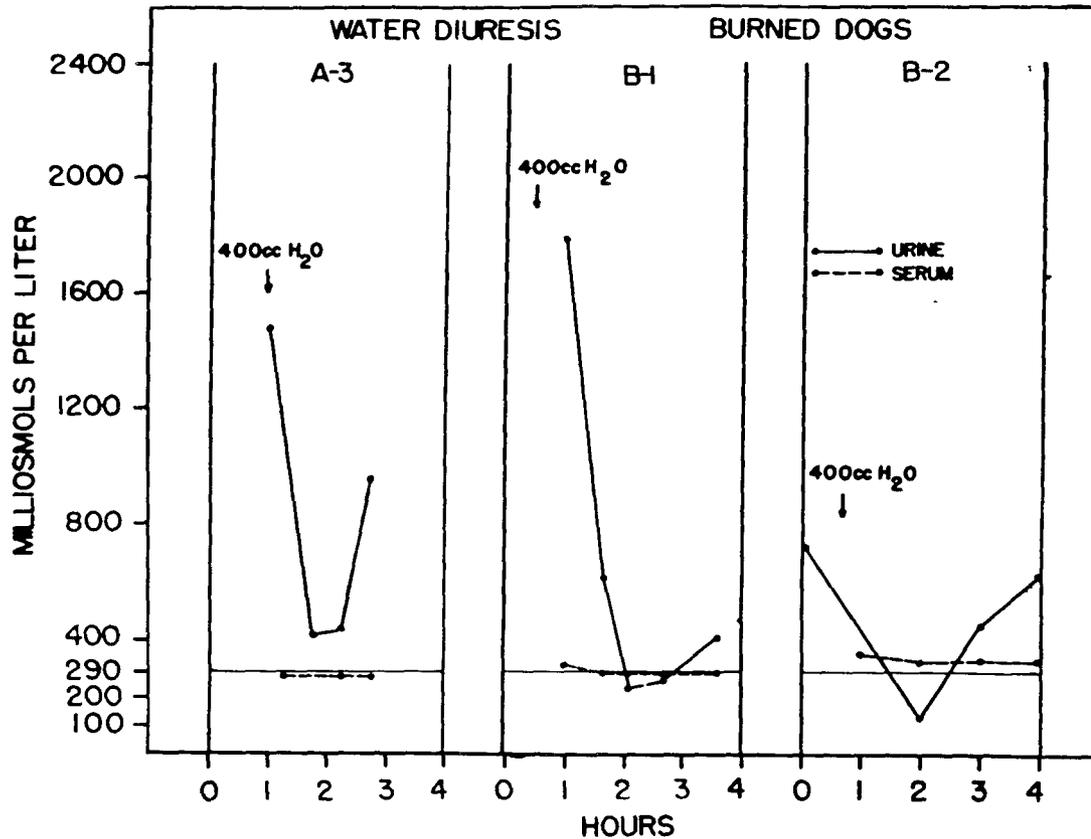


CHART II. Normal water diuresis in 3 of the 4 dogs under various circumstances in the post-burn period. Dog A-3 diureses normally with a low serum solute concentration, and a thiocyanate space 111% of the pre-burn value; B-1 diureses normally with a normal serum solute concentration, a thiocyanate space 112%, and a blood volume 57% of pre-burn values; B-2 diureses normally with a high serum solute concentration, a thiocyanate space 136%, and a blood volume 125% of pre-burn levels. The observations depicted were between the 6th and 8th days after the burn.

Serum solute concentration at this time was nearly normal, however, varying between 300 and 292 milliosmols per liter during the period of observation.

Dog B-2, the second high replacement animal, underwent a normal water diuresis when the thiocyanate space was 136% and the blood volume 125% of pre-burn values and the serum solute concentration in the range of 350 to 323 milliosmols per liter.

The range of urine solute concentration on the day of the burn has been fixed in all but one of the animals at a level well below the concentrating ability displayed on the subsequent day (Chart I). Inasmuch as this represents an apparent waste of water at a time that the burn wound is expanding, this phenomenon is of interest. It has been found that an oral water load two hours after the burn will not lead to dilution of the urine. It is planned to administer pitressin at this time to determine the possibility of raising the urine solute concentration toward maximum levels.

This series represents an inadequate basis for any conclusions. Thus far, however, we have seen apparently normal water diuresis under fairly disparate conditions.

Of considerable interest to us is the narrow, fixed range of urine concentration immediately following the burn. This will be pursued further.

The investigation of this problem in burned patients is difficult. Multiple venipunctures, frequent, large water loads, and manipulation of electrolyte intake may all be impossible in the critically ill.

The first phase of the work with patients has been essentially descriptive. The variations in urine solute concentrations have been mapped in six patients. Intakes and outputs were recorded and serum solute concentrations drawn whenever possible. The range of urine solute concentrations observed was roughly between 300 and 800 milliosmols per liter with the extremes at

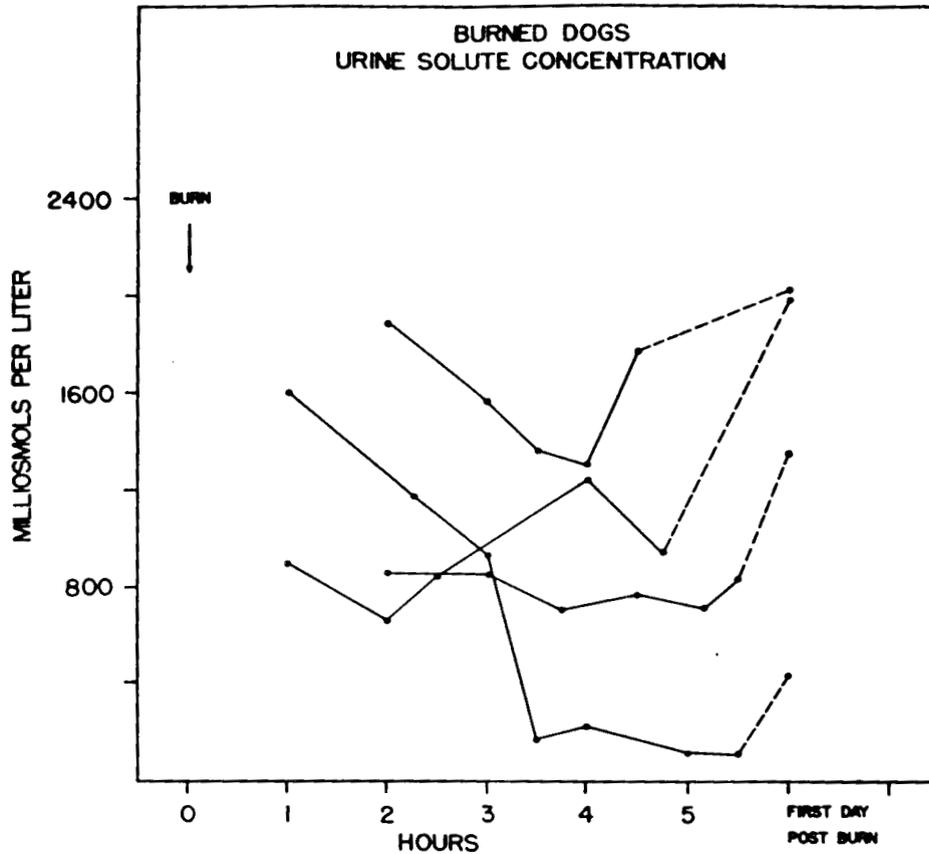


CHART I. Urine solute concentrations in 4 dogs burned 50% of their surface in water at 70° for 20 seconds. For therapy A-2 received 200 cc normal saline, A-3 none; B-1 and B-2 received 420 and 526 cc normal saline respectively in the periods depicted. The dogs were anesthetized with 1% chloralose, receiving a total of approximately 150 cc. All were subjected to a water load of 200 to 350 cc through a stomach tube 2 to 3 hours after the burn. It will be seen that no urine achieves maximum concentration in the hours immediately following the burn. With one exception, the urine solute concentrations observed the next day are substantially higher. Since the urine volumes were low, the findings suggest a temporary impairment of concentrating ability in the immediate post-burn period.

210 and 1137 milliosmols per liter. The normal range is thought to be 200 to 1200 milliosmols per liter. It is of interest that urine solute concentration rarely exceeded 900 milliosmols per liter.

One patient developed an enormous thirst, polydipsia, and polyuria about the 20th post-burn day. Fluid intake reached seven liters and urinary output five liters. Two previous water loads had been done, one intravenous, the other oral. The first was equivocal, but the second seemed to result in an entirely normal water diuresis. During the height of the polydipsia a Carter-Robb test was performed with the intravenous infusion of 2 cc $\frac{3}{4}$ % saline per kilogram body weight in 45 minutes. This raised the serum solute level from 268 milliosmols per liter to 276, enough in the normal person to cause a marked concentration of the urine and a marked fall in urinary output. There was no change, however, in the urine solute concentration of 400 to 600 milliosmols per liter and the urine volume actually doubled during the test. This indicated an impairment of the normal pituitary-renal water conserving mechanism. Intramuscular pitressin tannate in oil was given to determine the ability of the tubular mechanisms to reach to ADH. There was no clear cut response to this, indicating that renal tubular impairment was an important factor.

One explanation we considered for this "renal diabetes insipidus" was that the high calcium diet and immobilization may have led to high renal calcium excretion, a situation believed by Baker to interfere with renal water resorption. The patient died before further observations could be made.

The next patient (Chart III) was started on a low calcium regimen (less than 600 mgm. per day) and received a water load, a Carter-Robb test, and a pitressin injection consecutively in one 24 hour period. These studies were repeated in three weeks after the patient had been shifted to a high calcium regimen (3 to 6 gm. per day). On both occasions (Charts IV and V) there was a

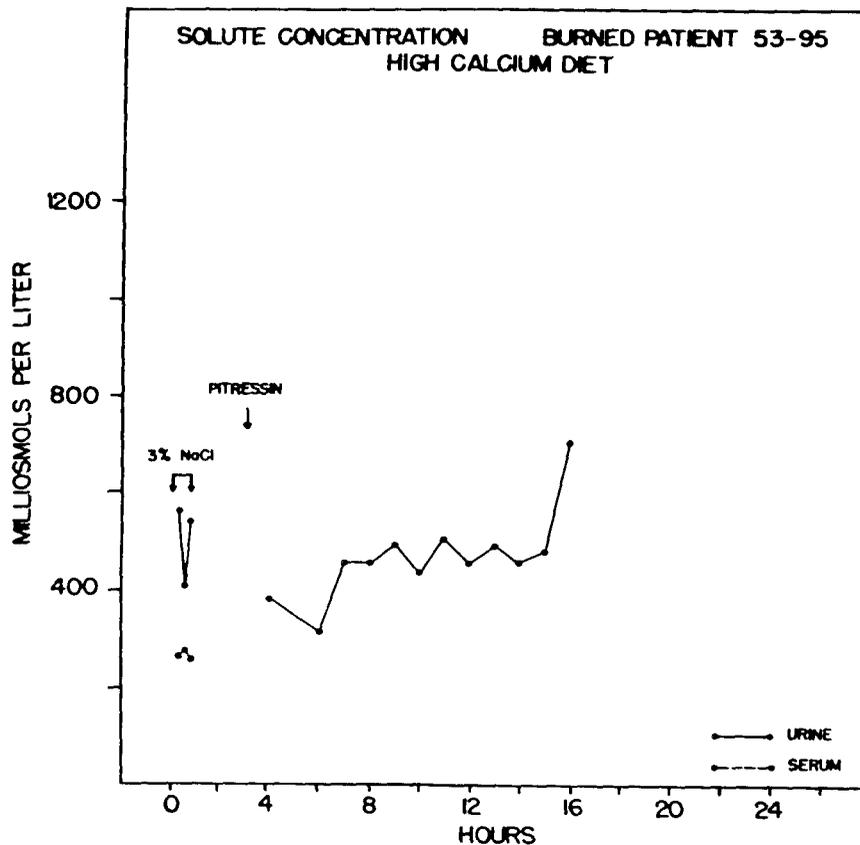


CHART III. The response to intravenous hypertonic saline and to 5 units pitressin tannate in oil in a severely burned woman of 43 on a high calcium intake(6 grams per day for the previous 3 weeks)(Case 53-95). The observations were made on the 22nd post-burn day during a phase of polydipsia and polyuria averaging more than 7 and 5 liters respectively for the previous 6 days. There is no significant urine solute concentration in response to either stimulus although there is somewhat higher concentration after pitressin than after saline. All values lie far below maximum urine concentration in the normal human being.

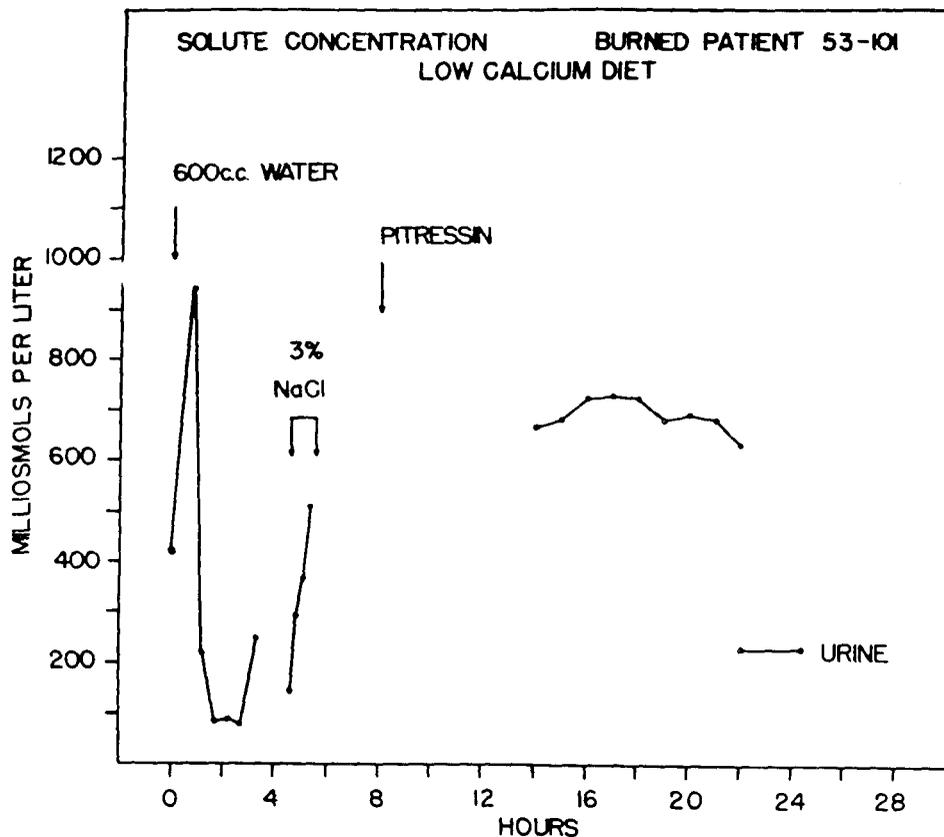


CHART IV. The responses to oral water, intravenous hypertonic saline, and intramuscular pitressin tannate in oil in a severely burned woman of 42 are shown (Case 53-101). The observations were made on the 12th post-burn day. The patient had had an intake of less than 600 mgm. calcium per day for the 2 preceding weeks. A normal water diuresis occurred. There is a sharp rise in urine solute concentration in response to the infusion of hypertonic saline, but this rise falls far short of the level of maximum concentration in the normal human being (1200 milliosmols). Pitressin results in higher urine solute concentration but again one far below normal maximum capacity.

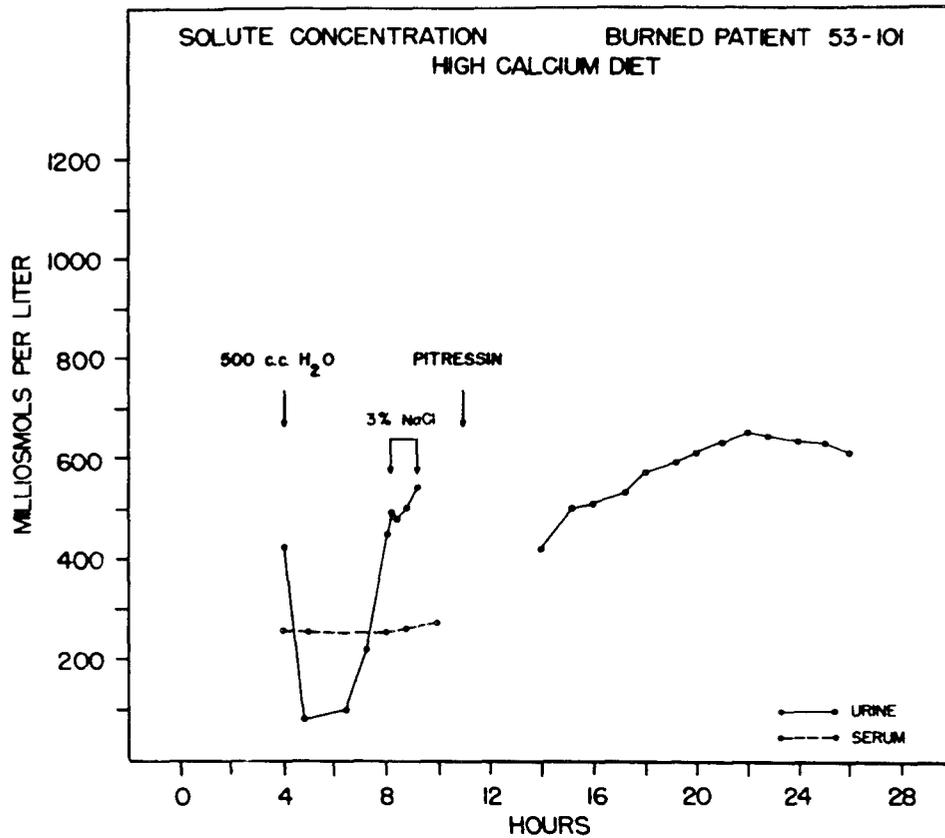


CHART V. The responses to oral water, intravenous hypertonic saline, and intramuscular pitressin tannate in oil in the same patient as Chart IV after 3 weeks of an intake of 3 grams of calcium per day (Case 53-101). It will be noted that there is no essential difference from the responses on the low calcium regime. Water diuresis is normal, and urine solute concentration is far below normal maximum levels in response to hypertonic saline and pitressin. Again the urine is somewhat more concentrated following pitressin than following hypertonic saline.

notable inability to concentrate the urine maximally in response to the pitressin or saline though a considerable dilution was noted each time in response to a water load. The maximum concentrations during the Carter-Robb tests were 510 and 543 milliosmols per liter respectively, following the pitressin injections 732 and 657 milliosmols per liter respectively. It will be noted that these values all fall considerably below the maximum concentration seen in the normal human being. It may be significant that there was a greater response to pitressin than to the elevation of serum solute levels with the Carter-Robb test; this would indicate a degree of posterior pituitary insufficiency.

Further observations are being made.

THE PHYSIOLOGIC DERANGEMENTS AND DEFICITS IMPERILING THE PATIENT WITH
A PERFORATED PEPTIC ULCER

I. The Dehydration and Fluid Shifts

by

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These studies were aided by financial support from Departments of the
Army and Navy, NR 114-198

INTRODUCTION

Abundant evidence has accumulated in recent years to indicate that surgical diseases and operations give rise to widespread and significant metabolic derangements*.¹⁻¹² A number of conditions have been investigated, and a general pattern is emerging, but the differences between individual conditions or operations which may be of significance to therapy are not fully apparent. Only in a few areas, such as burns and fractures, has a single entity been studied repeatedly, and in both of these extensively investigated conditions the variations within the group are wide. To date the experience with the metabolic changes of surgical disease might be called horizontal. Five years ago it occurred to us that it would be valuable to take a more vertical view, studying one entity sufficiently often to encounter most of the variables. We hoped this type of investigation would enable us to describe with authority any common pattern which emerged and would lead to a more secure plan of therapy.

The basic requirement for selection of the surgical condition to be studied was that it should be a disease entity with a relatively uniform pattern of injury from patient to patient. The consequences of the perforation of a peptic ulcer fulfilled that requirement.

Three other factors also entered into the selection of perforated ulcers; the active controversy as to the management of the perforation, the high mortality rate irrespective of the therapy chosen, and the probable similarity of the injury to that of a cutaneous burn. At present three modes of therapy for perforation have enthusiastic supporters and no agreement exists as to which treatment should be employed. The conservatives avoid surgery, the middle-of-the-road surgeons advocate simple closure and the more radical would add immediate subtotal gastrectomy. Each method of treatment involves a risk.

*There are numerous papers covering this field of interest. Those quoted constitute a small but representative fraction of the existing literature on metabolic effects of surgical disease.

Simple closure is relatively well tolerated if performed early and it prevents extension of the chemical peritonitis by sealing off the perforation through which the irritating peptic juices escape. The risk of this operation increases, however, as the interval between perforation and operation lengthens.¹³⁻¹⁹

The decision to go beyond the immediate closure of the perforation adding subtotal gastrectomy offers the possibility of a healthier future at the risk of a more hazardous operation.

The conservative, non-operative approach is based in part upon the concept that the likelihood of spontaneous sealing of the perforation increases with the passage of time. A more important reason for abandoning operative therapy, however, is that the patient who suffers from this form of peritonitis sickens rapidly and after a few hours withstands operation poorly.

The mortality rate regardless of therapy is high. DeBakey reported an overall mortality of 23.7% in 2,746 cases studied in 1939.¹⁸ In the literature widely different mortality statistics are found for each of the three current methods of therapy. Death rates from 84% to 0 are given for non-operative management, from 65 to 2% for simple closure, and from 29 to 0% for gastrectomy.¹⁸⁻²⁷ A review of 289 perforations in this hospital between 1940 and 1951 revealed an operative mortality of 4% and a fatality rate of 41% in unoperated cases.²⁸ A multiplicity of factors, including selection of cases, availability of antibiotics during the study and postoperative management make comparison of mortality figures from different clinics and different periods unsatisfactory.

Because the problem of therapy cannot be settled by mortality statistics and because of the continued high mortality, it is evident that further investigation into the factors contributing to the deterioration of the perforated ulcer patient is needed. Some of the confusion about the choice of therapy might be dissipated if we knew more about the metabolic derangements and nutritional deficits contributing to that deterioration. Our selection of ulcer perforation for study was based in part

on the hope that our findings might not only be informative regarding injury and operation in general, but that they might lead directly to improvement in the management of the perforated ulcer patient and those with other intestinal injuries and perforations.

The perforation itself is a wound of minor importance. It is the insult of peritoneal contamination with gastric juices which constitutes the real injury, a chemical burn. The area of the peritoneal surface involved is probably wide enough to be equivalent to a skin burn of moderate extent. This comparison with a cutaneous burn is an obvious one and suggested the probability that the same methods of study which had contributed so much to the understanding of burn shock might yield valuable information about the effects of peptic ulcer perforation and any subsequent operation as well.

The study has confirmed the anticipations both in uniformity of findings and in similarity of the metabolic and nutritional changes to those seen in burned patients.

PLAN OF STUDY

The Material

To insure an adequate view of the pattern of injury and its complications the study included twenty-four patients. There was no selection of cases. The patients were consecutive in so far as the Surgical Metabolic Unit and Laboratory were able to accept patients for study. Treatment included simple operative closure in 23 cases. The interval between perforation and arrival at the hospital averaged 3 hours and 9 minutes. There were no primary resections. The anesthetic agents employed were ether and nitrous oxide in 21 cases and spinal pontocaine in 2. One patient was managed conservatively without operation. He did not reach the hospital until 11 hours after perforation. Twenty of the patients ran relatively uncomplicated courses. Four required secondary operations. There were no deaths. Chance dictated that all the patients should be males. No females with perforated ulcers presented themselves for

admission to the hospital during the period of our investigations. The age range was from 31 to 69 years, the average being 48. The patients' average weight on admission was 57.8 kg. (127.2 lbs.). The location of the ulcer was duodenal in 15 cases, gastric in 8, and undetermined in 1, the unoperated case.

The operative and post-operative management were those current in this hospital at the time and were ordered and carried out by the resident staff. The blood pressure was determined on entry, every 5 to 10 minutes during the operations and every half hour for 8 to 12 hours thereafter. Post-operative treatment consisted of sedation, naso-gastric suction, intravenous dextrose, saline and whole blood as needed. No plasma was given. Although the fluid therapy varied from patient to patient, on the average the patients received during the first three days a total of nearly 7,000 cc of non-electrolyte glucose solution and 3,500 cc of saline. Throughout this period all were on continuous naso-gastric suction and were given only sips of water by mouth. The adequacy of the fluid therapy was judged by means of the urinary output. Thirteen patients were transfused either at operation or when the degree of anemia present warranted whole blood therapy. On request from the research team supplementary nitrogen, potassium and phosphorus were given in special cases. Gastric drainage was discontinued and oral feeding resumed between the third and eighth day after perforation at the discretion of the surgical resident. The time of ambulation was not fixed.

Investigative Routine

The investigative routine was oriented to observe any shift in water, electrolytes or nitrogen which might occur within the body or between the body and its environment as a result of the peritoneal irritation following perforation or as a result of operation and the subsequent surgical care. From the moment of admission to the hospital until the day of discharge the patients were kept under metabolic scrutiny. Immediately following establishment of the diagnosis and prior to the onset of fluid therapy in the majority of the cases the patient was weighed, blood samples were drawn

for serum protein and electrolyte determinations and an indwelling catheter was inserted in the bladder. The volume and specific gravity of the urine were measured hourly until good renal function was assured and the adequacy of the rate of therapy established.

A special effort was made to collect the urine excreted between the time of perforation and the onset of fluid therapy. It seemed likely that in this period before correction of the patients' dehydration any shifts of electrolytes from the cells to the extracellular fluid and from the extracellular fluid through the kidneys to the urine would be most easily observed. Thereafter all urine, feces, vomitus, and gastric drainage were carefully collected for quantitative analysis. In 4 cases fluid aspirated from the peritoneal cavity at operation was also saved for study. The only unrecorded fluid shifts between the patients and their environment were losses through the skin and respiratory tract. To assure adequate records of fluid intake and output, patients were moved immediately after operation to the Surgical Metabolic Ward under the supervision of a specially trained Metabolic Nurse.

The shifts of water within the body were measured directly by repeated determinations of the extracellular fluid and plasma volumes, and indirectly by measurement of changes in the hematocrit and the electrolyte and protein concentrations of the blood serum. Pre-operative determinations of the plasma volume and the extracellular fluid volume were carried out soon after admission. The measurements were made before fluids were administered unless the patients were sufficiently ill to make immediate fluid therapy mandatory.

The electrolyte shifts within the body and losses from it were also studied by observing the total electrolyte balances. To separate the shift of ions due directly to redistribution of water in the body compartments from that due to secondary metabolic derangements, comprehensive metabolic studies were made.

This first paper reports the dehydration which follows on the heels of the perforation. The subsequent papers deal with the shifts in electrolytes and nitrogen.

METHODS

The plasma volumes were measured by the dilution of Evans Blue Dye, T 1824.²⁹ The red cell volume was calculated from the plasma volume and the large vessel hematocrit. By the dilution of the thiocyanate ion³⁰ and radioactive sodium ion (Na^{24})³¹ measurements were made of the volume of fluid into which those ions passed freely and rapidly, a volume commonly thought to be the volume of the anatomic extracellular fluid space (see below). The normal values for these volumes have been found in this laboratory to be as follows:³²

Thiocyanate space	18.0 - 25.0%	Body weight
Sodium space	18.0 - 25.0%	" "
Plasma volume	3.5 - 4.5%	" "
Red cell mass	3.0 - 4.0%	" "

OBSERVATIONS

1. Hematocrit Changes: Initial hemoconcentration and subsequent dilution with fluid therapy

Changes in the hematocrit were followed in 23 patients. In general measurements were made once or twice a day on the day of perforation and the first postoperative day, daily for two or three days thereafter and then at gradually increasing intervals. On admission nine patients had hematocrit levels above 50%. In only two cases (cases 9 and 20) were sub-normal levels encountered and in those cases hemoconcentration was almost certainly masked by a pre-existing anemia. Although levels of 42 to 50% are accepted as normal for the hematocrit in the male, in patients with peptic ulcers downward deviations due to anemia are to be expected. Hematocrits above 50% may be considered clearcut evidence of vascular dehydration. The hematocrit changes of the first 10 patients studied are shown in Chart 1A.

After admission the hematocrit levels fell in 20 of the 23 cases. This drop coincided with the administration of parenteral fluids. The three cases whose hemato-

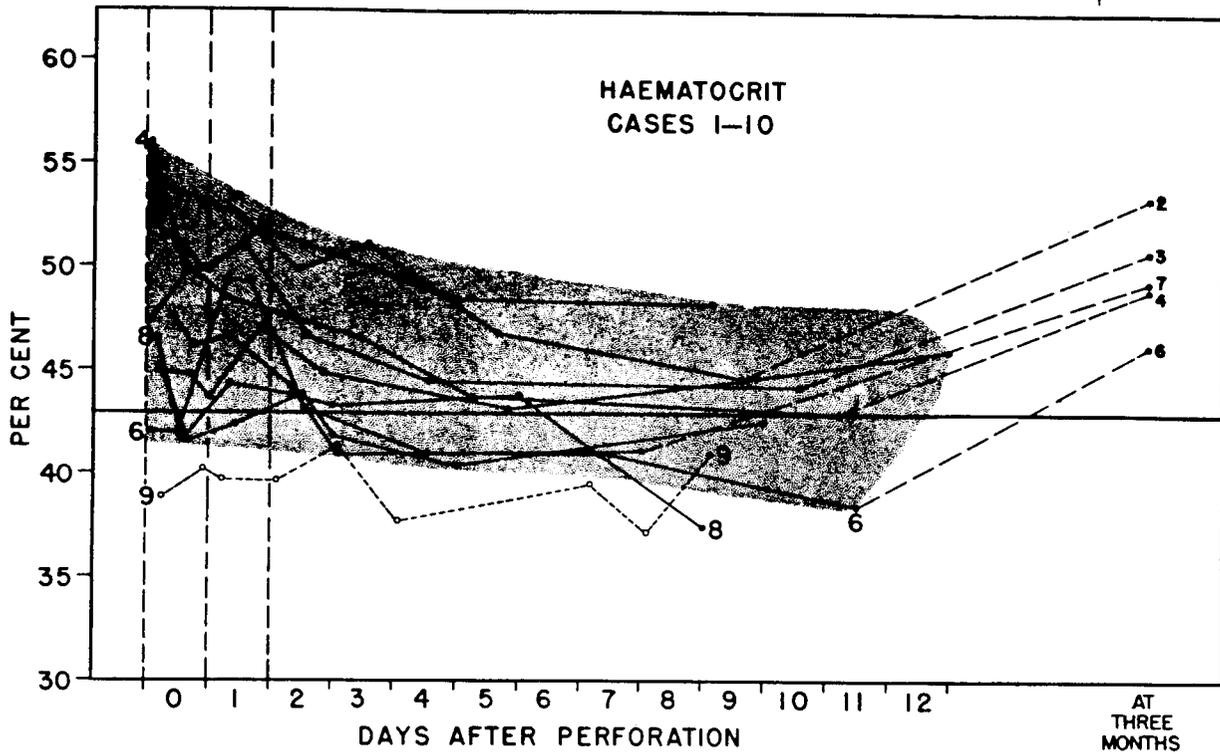


CHART I. EVIDENCE OF HEMOCONCENTRATION IN THE TRAUMATIZED PATIENT.

- ▲. Elevation of the hematocrit due to early dehydration in the perforated ulcer patient. The dotted line, the lowest, shows the levels of a patient with pre-existing anemia. This patient gave a history of at least one massive gastrointestinal hemorrhage prior to perforation. Another hemorrhage occurred on the tenth postoperative day, requiring subtotal gastrectomy.

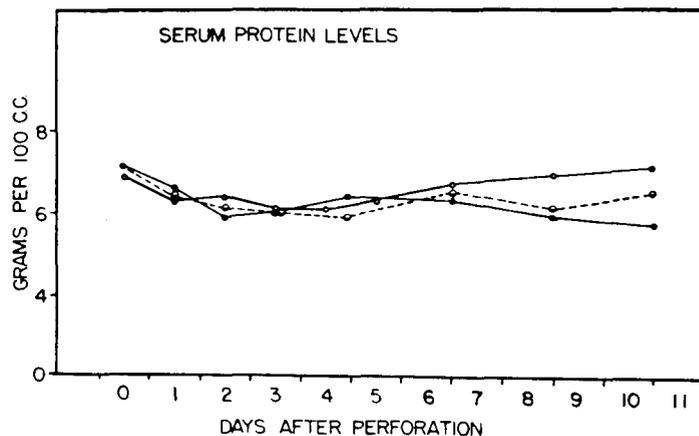


CHART II. ELEVATED INITIAL SERUM PROTEIN CONCENTRATION AND SUBSEQUENT FALL AS DEHYDRATION IS RELIEVED BY FLUID ADMINISTRATION.

The open circles indicate the protein concentration in patients receiving a low nitrogen intake and the filled circles indicate those receiving a moderate or high nitrogen intake. Parenteral nitrogen therapy did not prevent the drop in serum protein concentration. The effectiveness of intravenous amino-acid therapy in preventing nitrogen loss is discussed in the paper on nitrogen.

secondary changes were encountered.

A. The Plasma Volume

(1) Initial Contraction. Initial plasma volume determinations were made before any appreciable amount of fluid had been given in 10 of the 21 patients whose plasma volumes were measured. Six of these volumes were smaller than normal.* Four of the 11 cases who received fluid therapy before the first measurement also showed an initial reduction in the volume of plasma. In 12 patients, including 6 of those measured before treatment, two determinations were made within the first 36 hours after perforation. A reduction in plasma volume occurred between the two measurements in 4 of the 12 cases (Chart III). The average fluid intake in the interim was 2,154 cc. This group of 4 included 2 of the patients who had failed to show contraction of the plasma volume before fluid therapy. These 2 patients were among those reaching the hospital relatively soon after perforation, (1 and 2 $\frac{1}{4}$ hours). A fifth patient showed no alteration in plasma volume during the early hours.

(2) Subsequent Expansion with Fluid Therapy. In the remaining 7 patients whose volumes were measured twice in the first 36 hours, expansion occurred. These patients had an average fluid intake of 3,208 cc (Chart IV). As the volume of infused fluid increased, re-expansion eventually occurred in all 18 of the patients on whom repeated determinations were made. The average volume increase was 0.6 liters with a range from 0.2 to 1.6 liters.

B. The Interstitial Fluid Volume

(1) Initial Expansion. At the time when the plasma portion of the extracellular fluid was contracted, the total extracellular fluid volume was expanded (Chart IV). On the day of perforation thiocyanate volumes were measured in 20 of the cases treated surgically, 4 of them also having radioactive sodium space measurements. The thiocyanate volumes averaged 16.1 liters, exceeding the patients' expected normal

*Since the "normal" volumes were calculated on the basis of the low admission weights these decreases in plasma volume were all the more significant.

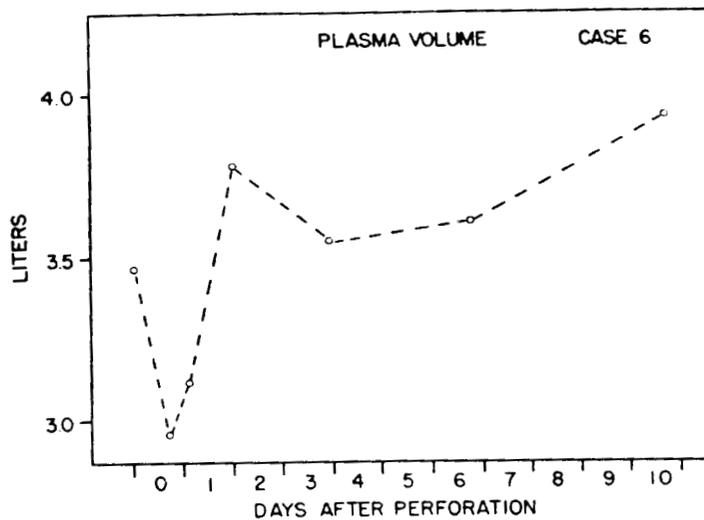


CHART III. CONTRACTION OF THE PLASMA VOLUME AFTER ULCER PERFORATION: RE-EXPANSION WITH FLUID THERAPY. (Case 6).

- A. The plasma volume changes. Despite receiving 2,942 cc of fluid in the 18 hour interval between the first and second blood volume measurements, the plasma volume of this patient diminished. When an increase in the rate of fluid administration in the next 9 hours brought the total intake to 5,400 cc the plasma volume began to re-expand.

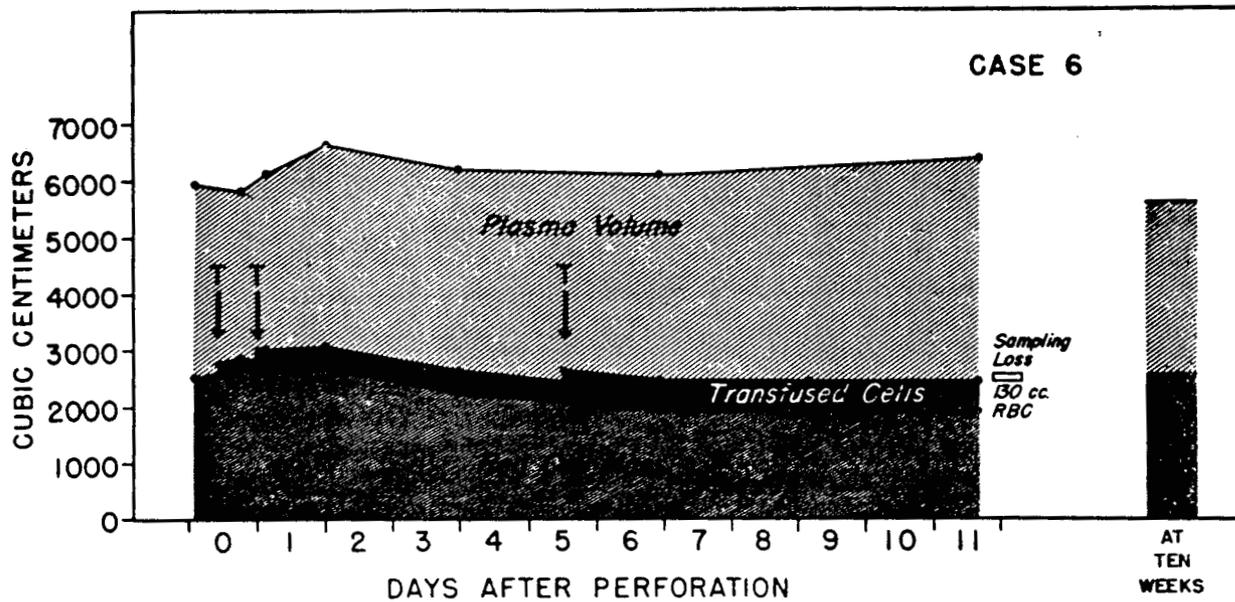


CHART III. CONTRACTION OF THE PLASMA VOLUME AFTER ULCER PERFORATION; RE-EXPANSION WITH FLUID THERAPY. (Case 6)

B. Whole blood volume and red cell mass changes. In addition to requiring fluids to restore his plasma volume the patient also needed 3 whole blood transfusions, indicated by the arrows, to maintain his red blood cell mass.

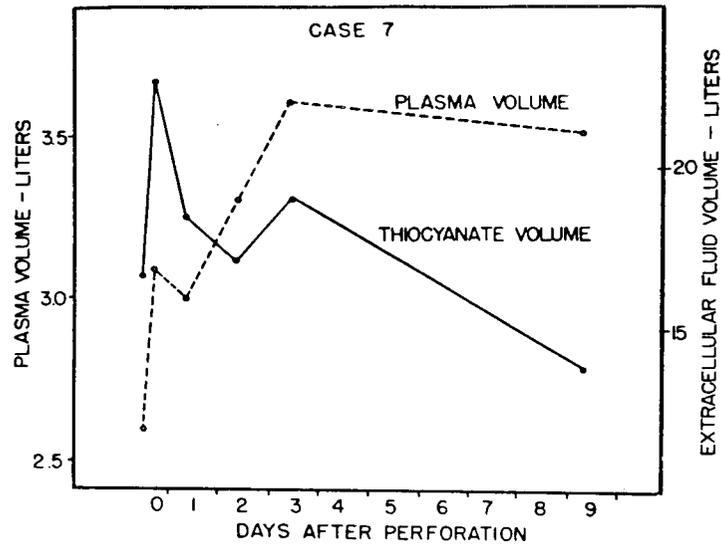


CHART IV. INITIAL CONTRACTION OF PLASMA VOLUME AND EXPANSION OF THE THIOCYANATE VOLUME AFTER TRAUMA; SUBSEQUENT EXPANSION OF BOTH WITH HYDRATION. (Case 7)

On admission this patient had a subnormal plasma volume and a greater than normal thiocyanate volume; both spaces expanded on fluid therapy.

9
~~10~~

spaces by 3 liters.* The average sodium spaces showed a 2 liter increase over the expected volume.

(2) Further Expansion with Therapy. Coincident with the re-expansion of the plasma volume, the already expanded thiocyanate and radioactive sodium volumes increased still further (Chart V). Peak volumes were reached at intervals from 4 to 64 hours after perforation, the average being 28 hours. Due to the irregular availability of Na^{24} insufficient data were collected to permit averaging of the sodium space expansions, but peak volumes of 19, 21, and even 24.5 liters were obtained. Sodium space expansions greater than 4 liters were encountered in 6 patients and the thiocyanate expansion exceeded this range in 19 cases. The average of the peak thiocyanate spaces of the 21 cases reached a volume of 20.6 liters, 4.5 liters above the average admission volume and 7.4 liters in excess of the expected size of the extracellular fluid space (Range 2.9 - 11.9 liters).** The measured increases between admission and peak thiocyanate concentrations were greater than 6 liters in 5 cases and above 4 liters in 3 others. These expansions are too great to be explained by the experimental error inherent in the thiocyanate and sodium methods.

(3) Final Contraction. After the rapid initial expansion the thiocyanate volume contracted slowly toward normal in patients in whom wound healing and recovery were progressing satisfactorily (Chart V). Final volumes obtained during the second week after perforation in 15 cases averaged 16.1 liters or 28.3% of their body weights. Four patients, whose spaces were measured 3 to 6 months after recovery, showed a subsequent further drop of from 1.1 to 3.5%. It should be emphasized that a smooth

*The expected volumes are calculated from the patients' admission weights, assuming the volume of extracellular fluid in liters to be equal to 22% of the body weight in kilograms. We have found that in normal subjects the volumes of the thiocyanate and sodium spaces are close to that percentage of body weight uncorrected for specific gravity of the fluids involved.

**Evidence that the apparent extracellular fluid space expansion is not an artefact due to use of the patients' low admission weights as a basis for calculation of the expected normal spaces is found by substituting 15.4 liters, the normal extracellular fluid volume of a 70 kg. man, as a base line. The average expansion then becomes 4.9 liters with a range from 0.9 to 8.4 liters.

pattern of initial expansion and subsequent contraction of the thiocyanate space was not obtained in all cases. Many unexplained drops and rises occurred.

C. Secondary Expansions in Patients Developing Complications

In 2 of the 4 patients whose recovery was delayed by complications requiring additional surgery, the return of the extracellular fluid volume to normal was deferred. In one of the 2, Case 23, wound dehiscence and operative closure were followed by a secondary expansion of both spaces. Four days after the procedure the thiocyanate volume reached 27.2 liters, the largest expansion observed in any case. Radioactive sodium was not available at the time of the second thiocyanate peak, but on the preceding day a determination showed a rise to 20.7 liters. In the second patient, Case 18, the thiocyanate space, instead of reaching a peak in the first day or two after perforation, expanded steadily for the first 9 days (Chart VI). This rise coincided with the development of a subdiaphragmatic abscess. While the thiocyanate volume soared to 10 liters above the expected extracellular space, the radioactive sodium volume failed to do so. Essentially normal volumes of 11.3 and 11.5 liters were observed on the eighth and ninth days when the thiocyanate volumes were 17.9 and 20.1 liters. The difference between the two spaces is well beyond the limits of error of the two methods. A second minor rise in thiocyanate volume followed surgical drainage of the abscess, and a third operation for pyloric obstruction 10 days later brought about a third expansion.

4. The Fluid Balance

A. Intake

The volume and nature of the fluids administered varied from case to case, but on the average the patients received 1,125 cc of saline and 2,400 cc of dextrose in distilled water during the first 24 hours. Ten received whole blood in addition (averaging approximately 500 cc) and 6 were given 50 to 100 cc infusions of a solution containing potassium and phosphate ions as well. (See the second paper in this series.) On the second and third post-operative days the glucose and saline intakes were slightly

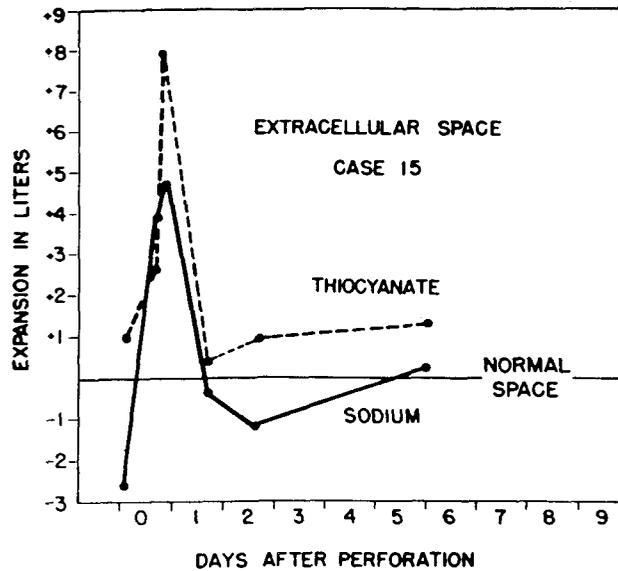


CHART V. EXPANSION OF THE EXTRACELLULAR FLUID VOLUME AFTER PERFORATION.

- A. Sodium and thiocyanate spaces in a patient whose course was uncomplicated. (Case 15). The extracellular fluid volume, as measured by thiocyanate and radioactive sodium ions, increased during interstitial edema formation and during rehydration of the initially depleted plasma volume. Contraction to normal accompanied wound healing and recovery.

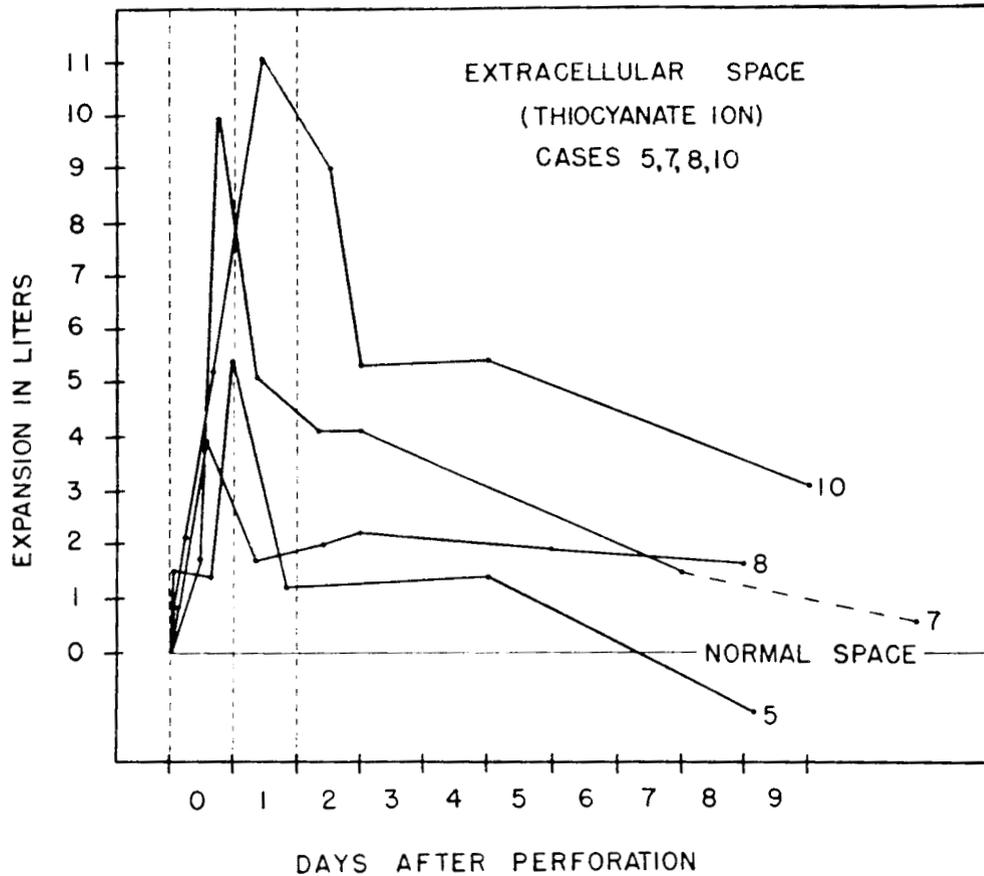
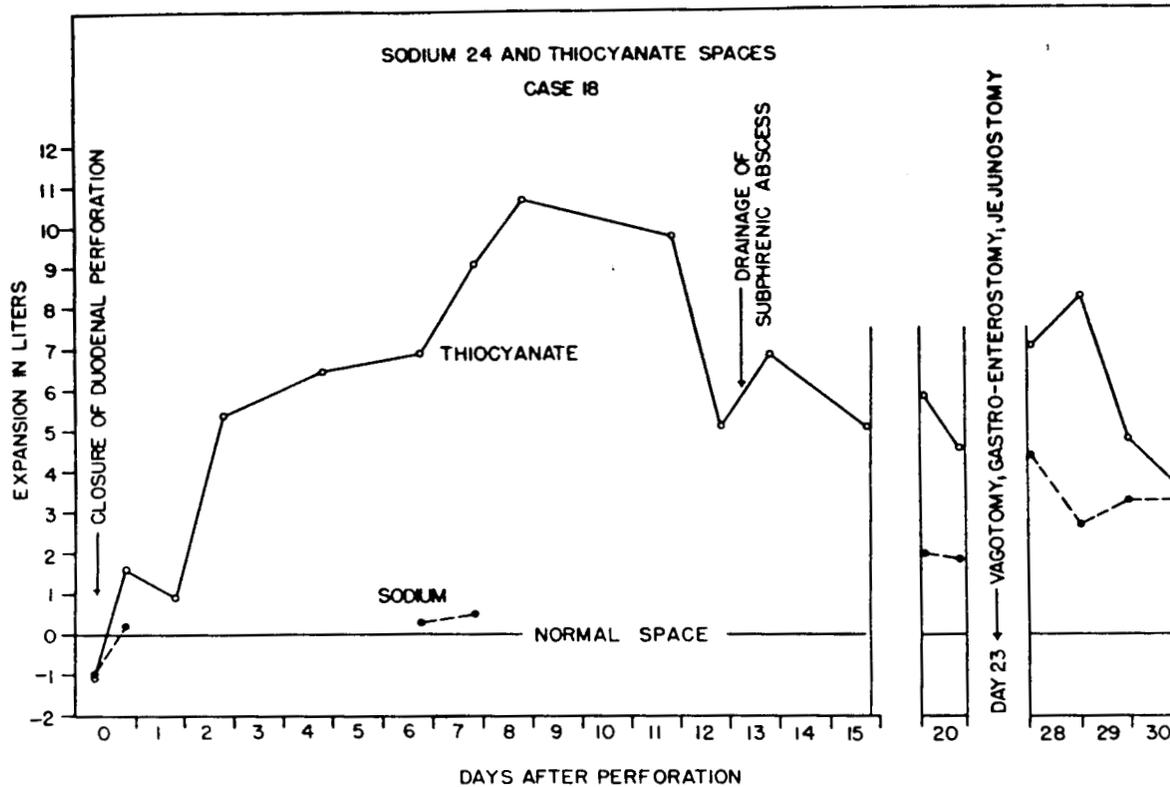


CHART V. EXPANSION OF THE EXTRACELLULAR FLUID VOLUME AFTER PERFORATION.

B. Thiocyanate spaces in 4 other uncomplicated cases. The increase in thiocyanate volume was rapid and exceeded clinical estimates of the volume of edema. The subsequent contraction of the thiocyanate space took place more slowly.



PART VI. PROLONGED ELEVATION OF THIOCYANATE SPACE IN PRESENCE OF SEPSIS; SECONDARY ELEVATIONS WITH ADDITIONAL TRAUMA. (Case 18)

The patient developed a subdiaphragmatic abscess which was drained on the thirteenth day. Gastroenterostomy, vagotomy and jejunostomy were performed 10 days later because of pyloric obstruction. The thiocyanate space rose with sepsis and after each operative procedure. The difference between the sodium and thiocyanate spaces is statistically significant, but not understood. Different cell membrane permeabilities to the two ions during sepsis may account for the discrepancy.

lower than the preceding day, averaging 1.9 and 1.8 liters of glucose in distilled water and 1.0 and 0.8 liters of saline. Thereafter, intravenous intakes decreased and oral intakes increased according to the tolerance of the patients.

B. Output

(1) Urinary Fluid Losses: Initially scanty outputs increasing on fluid therapy. Urinary outputs were measured in 18 patients. On admission the volume was scanty. The hourly rates of excretion prior to catheterization on entry were calculated whenever the patients were able to state the time of last voiding with certainty. Reliable information was received in 14 cases. Eleven were excreting less than 40 cc per hour. Of these 7 had an hourly excretion rate of less than 30 cc and 4 less than 20 cc.

During the early hours after admission urinary outputs continued low. The single patient treated without operation excreted no urine whatsoever for the first three and a half hours. Ten of the 18 operated cases put out less than 40 cc an hour in the first 3 hours after admission, 8 excreted less than 30, 4 less than 20, and 2 less than 10 cc, all of these in spite of intravenous therapy of from 62 to 263 cc per hour.

With continued fluid therapy, however, the urinary volume rose. Beginning the day after perforation the daily output averaged 2,000 cc.

(2) Gastric and Duodenal Losses. During the three day period of complete restriction of oral intake, gastric drainage and vomitus in our patients totaled from 80 to 3,298 cc per 24 hours, the average being 675 cc. With resumption of oral intake on the third post-operative day suction drainage was discontinued and either gravity drainage or intermittent aspiration substituted. On this regime and on fluid intakes increasing slowly from 30 cc of water per hour an increase in gastric output to nearly 900 cc resulted. The outputs fell to an average of 751 cc on the fourth post-operative day and by the following day half of the patients were showing so little gastric retention that their tubes were removed. Gastric

losses were prolonged in 4 patients with partial pyloric obstruction and also in the single unoperated case.

(3) Peritoneal Fluid Losses. The volume of fluid aspirated from the peritoneal cavity at the time of operation was not large. In 4 cases as much fluid as possible was removed by aspiration. The volumes were 38 cc, 125 cc, 200 cc, and 410 cc. The protein concentration of this fluid was in the vicinity of 4.6 gms. per 100 cc.

C. The Balance

During the day of operation and the first two post-operative days the fluid balances of all but 2 of the 18 patients for whom data is available were persistently positive. The volume of fluid retention was small, however, (average: 1.3 liters) and has not been corrected for respiratory or skin losses which would swing the balance to the negative side in at least 3 additional cases and possibly more.* Fluid balances for the succeeding days showed an uncorrected average daily positive balance of a liter.

Adequate fluid balance data are available in 13 cases whose thiocyanate volumes were measured repeatedly. Peak volumes in these cases averaged 20.2 liters, 7.1 liters above their expected normal volumes. The fluid retention between perforation and peak thiocyanate expansion in these cases averaged only 2.3 liters, 4.8 short of the apparent extracellular expansion (Chart VII). In only two cases could exogenous water account for the apparent increase in volume of the extracellular fluid. In 11, fluid balances fell short of the measured expansion by 0.1 to 9.4 liters. Fluid balances in 2 cases were actually negative at the time of peak expansion.

*Although pre-operative sweat losses may be high, post-operative losses are probably low. The work of Hardy and Ravdin on post-operative sweating³³ shows diminished sweat production in several post-operative cases including 1 after closure of a perforated ulcer. They found it difficult or impossible to obtain sweat from their patients in the early post-operative days. Furthermore, in our cases water of metabolism of foodstuffs and that water released by metabolism of the patients' own fat cells have been neglected in calculating the balances, creating a small error in the opposite direction.

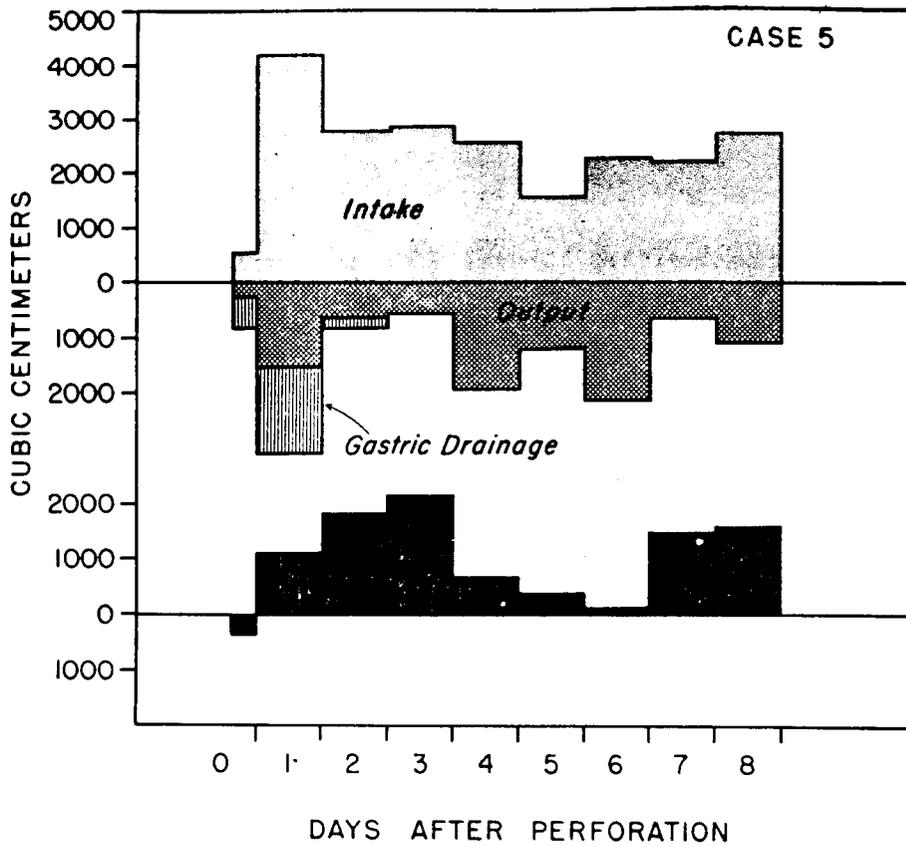


CHART VII. INADEQUACY OF FLUID INTAKE AS A SOURCE OF WATER FOR EXTRA-CELLULAR FLUID SPACE EXPANSION.

- A. Too small a positive balance to account for thiocyanate space expansion. (Case 5). His intake had exceeded his output by 1 liter at the time when his thiocyanate space had increased by 5 liters. (Chart V B).

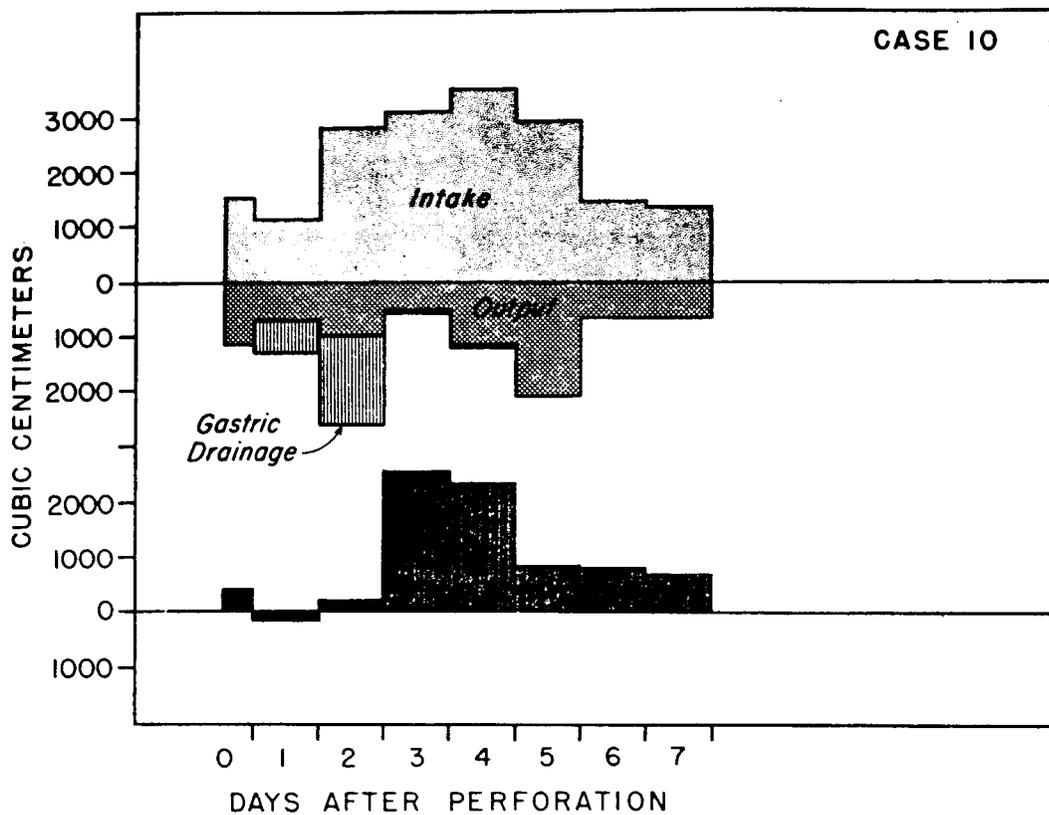


CHART VII. INADEQUACY OF FLUID INTAKE AS A SOURCE OF WATER FOR EXTRA-CELLULAR FLUID SPACE EXPANSION.

- b. Slight negative fluid balance in a patient showing an 11 liter thiocyanate space expansion. (Case 10). The 11 liter expansion in this patient (Chart V B) took place ~~on day 1,~~ in the first 36 hours during which his cumulative fluid balance was slightly negative.

5. Weight Changes

All 24 of the patients were underweight. Their first weights after admission averaged 57.8 kg. (Range 45.5 to 69.1 kg). In 9 cases body weight was measured twice within the first 36 hours. Gains were recorded in 4 which did not exceed 1 kilogram. Four others showed no change in weight despite measured increases in thiocyanate volume which in one case exceeded 9 liters. The ninth patient, whose thiocyanate space measured 15.3 liters on admission, lost 2 kilograms during the 37 hour period in which his thiocyanate volume expanded to 23.8 liters. After 36 to 48 hours a universal trend of gradual weight loss took place, halting only after oral feeding was resumed.

6. Blood Pressure Changes

Despite fluid therapy including, in 10 cases, transfusions of 400 to 500 cc. of whole blood, all 23 of the patients who came to operation exhibited operative or post-operative drops in systolic blood pressure averaging 30 mm. of mercury. The range was from 10 to 65 mm; the biggest drop was recorded in a hypertensive patient (Case 17). The hypotensive period lasted from 2 to 12 hours, averaging $6\frac{1}{2}$.

INTERPRETATION

The measurements of the redistribution of fluid in the patient with a perforated ulcer point clearly to an immediate and rapid vascular dehydration and less clearly to an expansion of the interstitial compartment of the extracellular fluid space at the expense of cell water. Since an understanding of such fluid shifts is essential to an enlightened fluid therapy, a critical interpretation of the measurements is mandatory.

The observations pointing clearly to an immediate and rapid vascular dehydration are sixfold: 1. the plasma volumes were contracted; 2. the hematocrits were high; 3. the serum protein concentrations were elevated; 4. the peripheral blood pressures were low; 5. the urinary volumes were scanty, and 6. all of these changes were reversed by fluid therapy.

This vascular dehydration was what we expected in the ulcer patients. The chem-

ical irritation of the peritoneum is like a burn. A wide surface is involved. Capillaries are damaged causing an outpouring of fluid which flows through the tissue spaces and seeps into the peritoneal cavity, joining that which has come through the perforation. The protein concentration of this free fluid is comparable to that of the fluid oozing from a burn wound (4.6 gm/100 cc). In both burns and perforated ulcers edema is formed by fluid from the plasma with reduction of the plasma volume and elevation of the hematocrit (Compare Charts 1 A & B). More water than protein escapes into the interstitial spaces with a resulting rise in the concentration of protein in the residual plasma.⁸ Hypotension and diminished urinary output are common to both injuries as plasma depletion reduces the effective blood volume.

It is possible that the fall in urinary excretion in these patients is due only in part to plasma loss with diminished glomerular filtration. The posterior lobe of the pituitary may also play a role in limiting urinary output.

The observations pointing less clearly to an expansion of the interstitial compartment with cellular dehydration are the measurements of the so-called extracellular space by the thiocyanate and sodium ions. Under normal conditions it is believed that the distribution of these ions is predominantly extracellular, only a small quantity penetrating cells. The remainder diffuses in equal concentrations through the vascular and interstitial fluids. Expansions of the thiocyanate and sodium spaces ranged from 2 to 11 liters. Exogenous water as the source of this expansion is excluded by lack of both an appreciable weight gain and a sufficiently large fluid intake. Either water must have been withdrawn from cells or the two ions entered spaces not normally open to them.

If it is assumed that the space measured by the thiocyanate and sodium ions is the extracellular space and that the water providing the expansion came for the most part from the cells, then a compatible quantity of intracellular electrolytes should have shifted to this space. That the shift of electrolytes observed was inadequate will be considered in the next paper.

If the thiocyanate and sodium ions measure the extracellular space then the expanded space in these patients must represent edema. Those who deal with cutaneous burns see large quantities of edema beneath and around the wounds. In our study of burn patients expansions of the thiocyanate and sodium spaces were observed which were considered consistent with the volume of wound edema together with the edema expressed or gravitating into the unburned areas.³² In the ulcer patients, however, the expansions far surpassed clinical estimates of the volume of edema which might puddle beneath the peritoneal surfaces or flow through the tissue spaces and lymphatics to points remote from the site of perforation. Expansions of two or four liters might be compatible with the volume of edema which the surgeon sees in the upper abdomen at operation. Sodium expansions larger than 4 liters were encountered in 6 of the patients studied, and the thiocyanate expansions exceeded this range in 19 cases.

A third argument against a large shift of intracellular water and in favor of the cell penetration concept is the disparity sometimes found in the volumes of the thiocyanate and sodium spaces. In normal individuals the two spaces are almost identical. In 6 of the 10 ulcer patients in whom both spaces were measured there was also no significant difference between measurements, but in 4 others there were unexplained discrepancies. An 8 liter difference between the two spaces was encountered in Case 18 during the phase of his subdiaphragmatic abscess (Chart VI). Here then are 2 ions, formerly thought to measure the same anatomic space, giving widely different values.

Comparable discrepancies between the thiocyanate and sodium spaces have been found in 2 of our burn patients. In these, as in Case 18 (Chart VI) there was widespread inflammatory edema. It is hard to believe that the inflammatory barrier alone is responsible for the discrepancy because in other burn patients with extensive inflamed wounds no disparity was observed (Chart VIII). The possibility that there may at times be different cell membrane permeabilities to the 2 ions offers the more acceptable explanation.

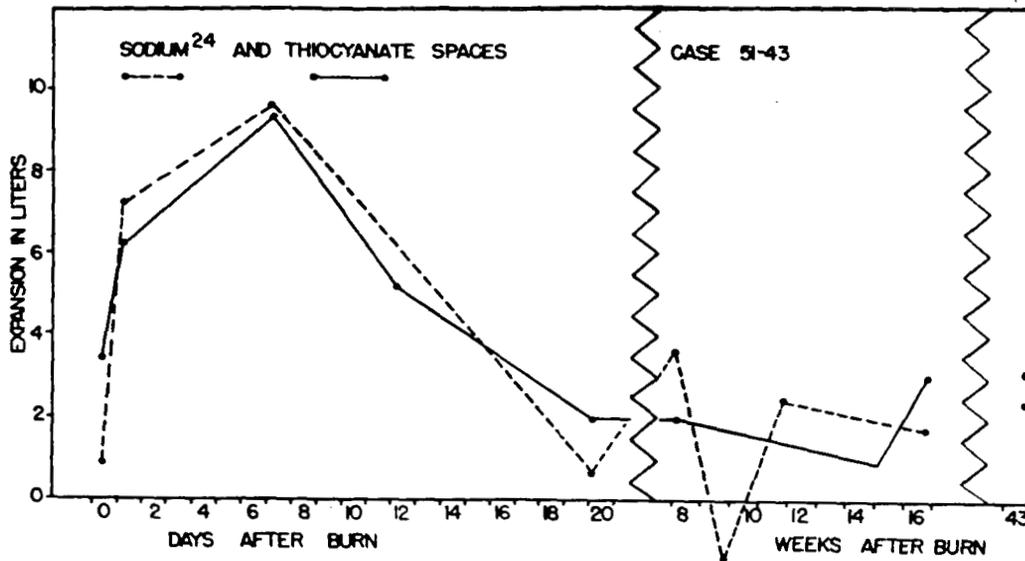


CHART VIII. EXPANSION OF THE EXTRACELLULAR FLUID VOLUME AFTER A SEVERE BURN.

The patient, a 30 year old male who survived burns of 68% of his body surface (30% of third degree), showed expansion of the thiocyanate space comparable to that seen in the patient with a subdiaphragmatic abscess after ulcer perforation. Sepsis was rampant at the time of peak expansion. In this case there was no discrepancy between the thiocyanate and radioactive sodium volumes.

The question of what the sodium and thiocyanate expansions mean is a practical one. If the extracellular space expands rapidly, depleting the cells of water, a large quantity of fluid should be infused in the early hours to combat cellular dehydration. If the expansion represents a change in cell permeability the giving of such an amount of fluid might be hazardous.

It has already been pointed out that a fuller interpretation of these fluid shifts must await consideration of the electrolyte data. Fluid shifts may also be influenced by colloids, which will also be discussed later. The plan for therapy emerging from these studies will be presented at the end of the fourth paper when all the evidence has been considered.

SUMMARY AND CONCLUSIONS

1. Perforation of a peptic ulcer is followed by immediate and severe dehydration. The chemical insult to the peritoneum is followed by edema and loss in volume of the circulating plasma. Sixfold evidence of this vascular dehydration is presented.
2. The magnitude of the fluid losses of the ulcer patient is described.
3. Fluid therapy replenishes the plasma and abolishes the other evidences of dehydration.
4. The so-called extracellular fluid volume measured by the thiocyanate and sodium ions expands after perforation, re-expands with complications and returns to normal with healing. The magnitude of the initial expansion exceeds clinical estimates of the volume of peritoneal edema. Two possible explanations are presented, each requiring a different fluid therapy. One is based on cellular dehydration and the other on a disturbance of cell membrane permeability.
5. Over-enthusiastic fluid therapy is excluded as the source of the apparent extracellular fluid expansion.
6. Further consideration of the magnitude of cellular dehydration and the volume of fluid required by the perforated ulcer patient is given in the subsequent papers.

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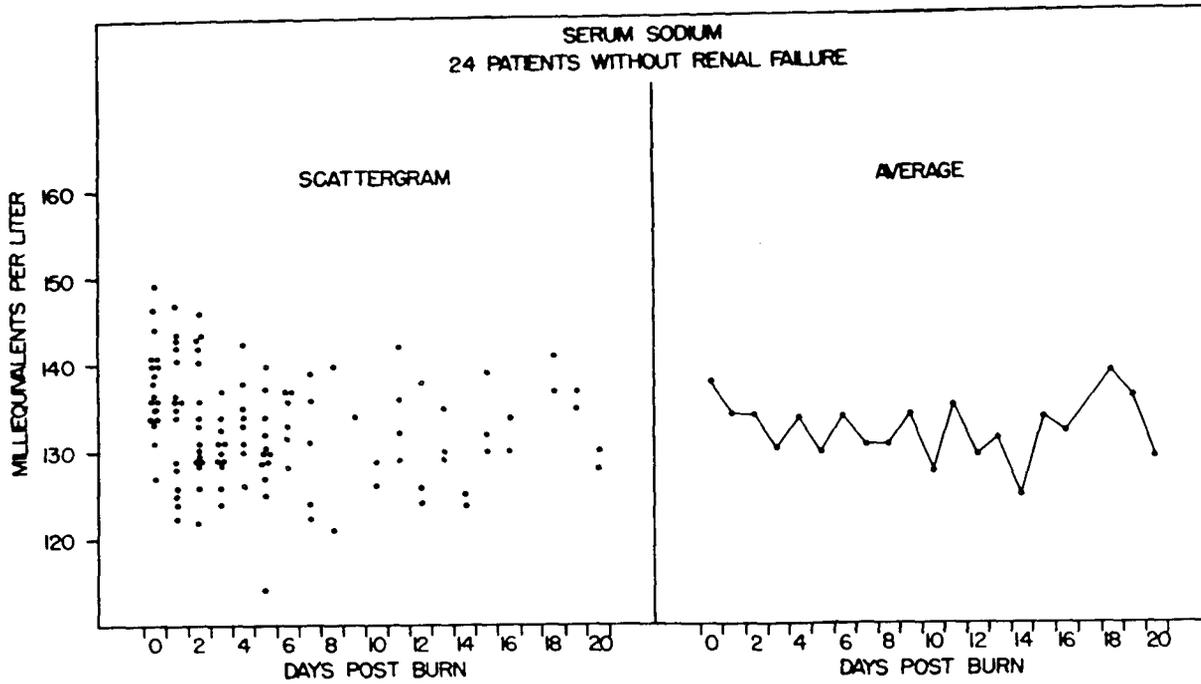


CHART A. Course of serum sodium in 24 severely burned patients who had no renal failure. The extent of body surface burned varied from 17% to 78%. Seven patients died of their burns which were from 35% to 70% of the body surface. The day of death ranged from the 7th to the 33rd day following injury. The remaining patients were eventually discharged healed. Sodium balances (calculated from intake of sodium minus the total of sodium loss in exudate, urine and stool) available on 9 patients were strongly positive during the period of burn shock therapy and then tended to be negative until the patient had begun to recuperate. The loss of sodium in the urine usually far exceeded that in exudate, and stool losses were consistently low.

The serum potassium level was also followed in 20 of these patients. It varied irregularly between 3 and 6 meq/l, averaging 4.3. There was no consistent pattern or variation with respect to the sodium level.

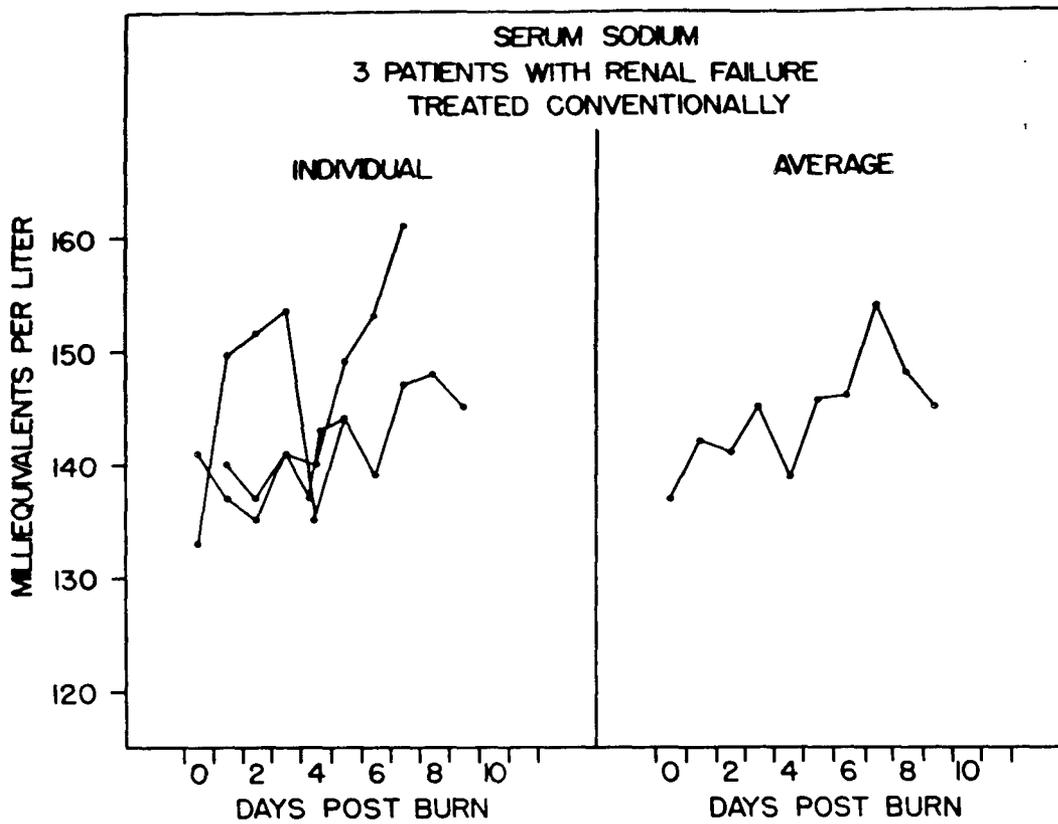


CHART B. Course of serum sodium in 3 severely burned patients with renal failure treated conventionally. The patients suffered burns of 55%, 68% and 85% of the body surface. They died on the 9th, 6th and 8th days post-burn respectively. Standard burn shock therapy on the day of injury consisted of 75 cc of both plasma and 0.85% saline for each percent of burn. Half of this volume was given during the next day. Each day 2000 cc of 5% dextrose in water is given to maintain imperceptible loss and urine volume. Sodium balances (calculated from intake of sodium minus the total of sodium loss in exudate, urine and stool) were completed on the latter 2 patients. After the positive phase during emergency therapy, they remained negative until the death of the patients. This occurred despite continuing sodium therapy. The cumulative sodium balances were plus 20 grams and minus 9.8 grams, the latter on the last patient who had the excessive rise in serum sodium to 161 meq/l on the day of death. The large majority of the sodium loss in these patients was in wound exudate rather than in urine, stool losses being consistently low.

The serum potassium level was followed in 2 of these patients. The level rose slightly in both. In the patient with the 68% burn it rose from 4.7 to 5.1 meq/l, in the 55% burned from 3.3 to 5.1 and then down to 4.3 before death.

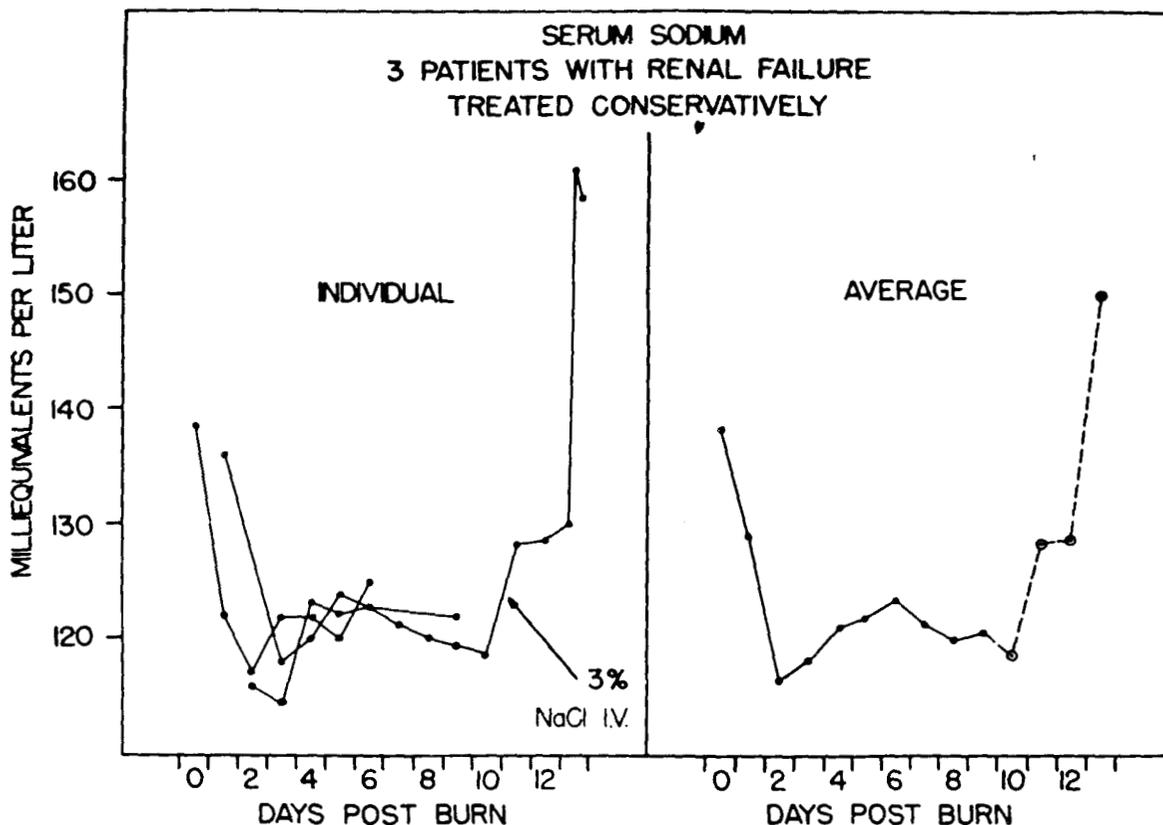


CHART C. Course of serum sodium in 3 severely burned patients with renal failure treated conservatively. The patients suffered burns of 30%, 45% and 62% of the body surface. They expired on the 13th, 7th and 13th days post-burn respectively. Standard burn shock therapy was given on the day of injury: 75 cc of both plasma and 0.85% saline for each percent of burn. Half of this volume was given during the next day. Each day 2000 cc of 5% dextrose in water is given to maintain imperceptible loss and urine volume. Thereafter these patients were given little sodium because of obvious renal shutdown except late in the course of one patient, who was given 3% saline solution. Sodium balance (calculated from intake of sodium minus the total of sodium loss in exudate, urine and stool) in this latter patient was negative only occasionally during his illness, and he expired with a cumulative balance of plus 30.9 grams of sodium. The other patients had negative sodium balances following the second day, but they died with positive cumulative balances of 14.9 grams and 6.5 grams. The large majority of sodium loss in these patients was in the wound exudate rather than in urine, stool losses being consistently low.

The serum potassium level was followed in 2 of these patients. In both in the first 5 days, it varied between 3.7 and 7.0 meq/l. In the one surviving until the 13th day it leveled off near 4 meq/l from the 6th day on. This was the patient receiving the 3% saline from the 10th to 12th days.