SOME ASPECTS OF NITROGEN METABOLISM IN THE RAT

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Thesis

submitted to the Faculty of

Graduate Studies and Research

in partial fulfillment of the

requirements for the degree of

Master of Science.

McGill University Clinic

Royal Victoria Hospital

Montreal, Canada.

September, 1945.

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

This investigation was carried out in the McGill University Clinic.
The author wishes to thank Professor J. C. Meakins for the provision of the facilities which made this work possible.
The author wishes to express his appreciation and gratitude to Doctor J. S. L. Browne for his untiring assistance and valuable criticism throughout the course of this research.
The author wishes to thank the Banting Research Foundation for financial assistance.

T A B L E O F C O N T E N T S

T A B L E O F C O N T E N T S (Continued

GENERAL INTRODUCTION

After damage there is a disturbance of protein metabolism the extent of which has been shown to be influenced by the previous state of health or nutrition of the animal. As yet the underlying mechanisms regulating these post-traumatic metabolic processes is unknown, but it has been suggested that the endocrine system, particularly the adrenal cortex may occupy an integral role.

The experiments to be described arose out of the observation by Cuthbertson that rats on a protein-free diet for 14 days did not show any increase in the excretion of urinary nitrogen after fracture of the left femur on the 14th day, while still eating the same diet; whereas, other rats eating diets of different protein levels did show an increase in the nitrogen excretion curve. In view of the findings in man, that accompanying the high output of urinary nitrogen after damage of a previously healthy individual was an increased output of gluconeogenic substances, presumably from the adrenal cortex, it was desired to investigate the possible role that the adrenal cortex might play in the metabolic behaviour after damage, of the animals eating the protein-free diet.

A review of those features of protein metabolism pertinent to the subject, follows together with a discussion regarding the behaviour of protein metabolism after trauma and the possible factors influencing this behaviour.

METHODS IN STUDYING PROTEIN METABOLISM

In 1842 Liebig (1) first suggested that urinary nitrogen might be used as a measure of the utilization of protein by the body. This approach consists in measuring the difference in nitrogen content of the ingested food and that of excreta (mainly feces and urine) and is still used as an approximation of the rate at which the organ handles protein. This method (nitrogen balance) only informs one as to the beginning and end of the metabolism of protein (mainly characterized by its nitrogen content) and other experimental approaches have been devised to gain insight as to the intermediary metabolism of protein.

As regards the feces, recent investigations (2, 3) have shown that the concept that fecal nitrogen consists largely of unabsorbed food residues, is incorrect. It is now generally accepted that fecal matter consists of:-

A large bulk of bacteria (approximately 9% of total solids), unabsorbed gastric, hepatic, pancreatic and intestinal secretions, leucocytes and desquamated epithelium of the gastro-intestinal tract, and although some investigators (4, 5) question whether the stool nitrogen can be considered as catabolic nitrogen in the same sense as urinary nitrogen, it is nonetheless generally conceded that in balance studies due consideration must be given to fecal nitrogen (4).

Various investigations have shown, that providing the composition of diet protein is such that it is capable

of being completely digested and absorbed (1, 3, 4) then the nitrogen of the stool is relatively constant in amount, although in certain conditions it may be considerably increased as in diarrhoea, where the fecal nitrogen consists not only of an unusually large amount of unabsorbed food, but also of an abnormal amount of intestinal secretions and debris.

Abnormally high fecal nitrogen may be expected in any condition which involves lesions in the gastro-intestinal tract. Recently Hoelzel and Da Costa (6, 7) have demonstrated the production of experimental ulcers in rats and mice by the administration of low or protein-free diet otherwise adequate, and Weech and Paige (8) reported similar findings in dogs. And therefore, in the experiments with low protein diets here one must bear in mind the possible double effect of the low protein diet, i.e., inadequate nutritive value per se and possible production of peptic ulcers leading to inadequate absorption and increased fecal excretion.

Abnormally high fecal nitrogen may be found in any condition involving failure of secretion by the glands responsible for the proper hydrolysis of protein. There is a recent report by Toerkischer and Wertheimer (9) in which they find marked diminution in gastric secretion with little or no free HCl and with low proteolytic enzyme content in adrenalectomized rats in good general condition, occurring 2-5 days after adrenalectomy, a state remedied by administration of cortin.

Generally it is common practice in balance experiments to estimate the fecal nitrogen as 10% of the total nitrogen intake.

OTHER METHODS FOR STUDYING INTERMEDIARY METABOLISM OF PROTEIN

Angiostomy:

A technique devised by London (10) who prepared fistulae of afferent and efferent blood vessels of different organs, mainly liver and kidneys, thus enabling one to analyze blood before and after it has left an organ which has previously been prepared in the above manner. Thus one can analyze samples of blood at will and also one can introduce substances and study, by analyzing blood samples leaving the organ, the manner in which the organ handles the introduced substance.

Growth:

Measure of growth in any organism may be used as an index of the manner in which protein is being utilized. Extensive studies having thus been carried out mainly in the rat by the fundamental work of Osborne and Mendel (11) and later in the classic studies of Rose (12).

Perfusion of Isolated Organs:

A procedure devised independently by Emden and Knoop some years ago - consists in studying an isolated organ, which has been removed intact and through which is circulated physiologically isotonic fluids which keep cells alive for some time. To this fluid may be added substance whose metabolism may be observed in perfusate. This method is one in which the organism is not operating in its usual in vivo environment and has thus been severely criticized by many.

Tissue Slice:

This technique, revised by Warburg, modified by later investigators, notably Dixon, consists in measuring, manometrically, the ratio O_2 consumption and CO_2 production of tissue slices immersed in a suitable physiological medium; the desired substrate may be added to this medium, and the effect on tissue respiration noted.

Plasma Pheresis:

In the hands of Whipple et al, this has become a valuable tool in studying at least the metabolism of one of the body's most important protwins, viz., plasma proteins, and will be discussed in fuller detail in section on "Protein Stores".

Isotopes:

Isotopes, chemical elements difrering from those commonly found only in atomic weight,have been shown to be treated in the same way as those compounds which possess natural form, when incorporated into the latter and fed to animals. By use of such "tagged" compounds, their course in the body is traced by determining the amount of isotope in the various tissues, either by mass spectograph which identifies the amount of heavy isotope or the Geiger Counter which differentiates compounds with radioactive isotopes and measures the latter.

Enzyme Isolation:

Certain aspects of protein metabolism have yielded fruitful results by isolating from tissues the enzymes involved, such as arginase in urea formation, but as yet this field is relatively undeveloped.

SIGNIFICANCE OF URINARY NITROGEN LEVELS AND FACTORS AFFECTING IT

Liebig (1) believing that protein contained nitrogen as an integral component, suggested that the urinary nitrogen might be used as a measure of the rate of protein destruction in the body. It was later found (3, 4) that the feeding of nitrogen substances (mainly meat protein) resulted in the excretion of about an equivalent amount of nitrogen, mostly as urea and ammonia, in feces and urine, and it was further established (15,16) that atmospheric nitrogen was not utilized by the body, nor was any "metabolic" nitrogen lost in respiration. Until the work of Schoenheimer, the prevailing view regarding the metabolic importance of urinary nitrogen was succintly stated by Lusk (17) "That urea, the principal end product of protein breakdown was shown to be not an adventitious product, but one normally proportional to the protein destruction".

However, with the demonstration of the constant dynamic interchange of chemical compounds within the body (18) where it has been shown that nitrogenous compounds found in the urine include representative of compounds both endogenous and exogenous in origin, one might well ask what does the level of nitrogen excretion actually represent? It might be that nitrogen which the body for some reason or other cannot retain, and as Schoenheimer has stated (19) "The nitrogen excreted by a normal animal in nitrogen equilibrium is a sample of the pool originating from the constant and rapid chemical interaction of food and body proteins", and it can be no more than the minimal level of catabolism of nitrogenous compounds.

In previous discussion evidence was presented which showed that the fecal nitrogen is relatively constant and within certain limits, independent of the nitrogen content of the diet and other extra-renal loss of nitrogen (sweating, hair loss, etc.) is not sufficient to significantly alter the total nitrogen excretion. In view of the above, one may consider that under most experimental conditions the urinary nitrogen as the only important variable moiety and one which would be expected to reflect the changes in nitrogen excretion under various conditions which affect nitrogen metabolism.

Folin (20, 21) in his extensive studies on the composition of human urine, first established the relationship between the quantity of nitrogen intake and composition of that excreted. From the above it became evident that in studies using urinary nitrogen as a metabolic index, due consideration must be given to the quantitative changes in nitrogen intake.

Protein (consisting of different amino acids) performs certain functions relating to growth and maintenance, functions which no other nutrients are capable of carrying out.

That one of the limiting factors of the nutritional value of a protein appears to be in the nature of its amino content stems from the results of the intensive studies by Rose et al (22).

Rose, McCoy and Meyer (23) employed synthetic diets, the nitrogen of which was furnished by a mixture of crystalline amino acids. At first they found that a mixture of all of the

then known amino acids failed to produce nitrogen equilibrium. It was reasoned that one or more unknown amino acids were lacking, and studies along this line led to discovery of the amino acid, threonine, which, when added to the then mixture of crystalline compounds, induced positive nitrogen balance and growth. Continuing his research by altering the various components of this mixture, Rose (24) was finally able to clarify the amino acids with respect to their necessity for growth in the rat. The absence of one of the indispensable amino acids will block all protein synthesis, and a relative deficiency in such an amino acid will permit only a subnormal rate of synthesis.

Another important consideration in the evaluation of the nutritive value of any protein is its digestibility and the concomitant availability of its amino acids from the gastrointestinal tract.

Therefore, the degree to which amino acids of the intake fulfill the requirements of the body, will under certain conditions determine in part theamount of their nitrogen appearing in the urine.

Carbohydrate and fat are the main energy-producing substances in the food. Their relative or absolute lack results in an increased catabolism of body tissue protein or its precursors in order to supply energy which would otherwise be derived from carbohydrates and fat.

Metabolic observations on the effects of starvation have yielded much information on the "protein sparing" actions of carbohydrate and fat. The nitrogen excretion during starvation is thus almost three times higher than when no nitrogen, but sufficient calories, in the form of carbohydrate and fat, are supplied.

The following table containing data from an experiment if Cathcart's (25) illustrates this point.

	Urinary nitrogen (amount)
14th day of starvation	7.78
1st " on cream (300 cc.), starch (400 g.) diet	7.43
2nd " " " " " " " "	3.58
3rd " " " " " " " "	2.84

Here the nitrogen excretion of an individual who had been fasting for 14 days is 7.78 g. and is reduced to 2.84 g. after 3 days intake of 400 g. starch per day.

Carbohydrate exerts a specific "sparing action" quite apart from the fact that it furnishes energy, for the "sparing effect" exhibited by a given quantity of carbohydrate cannot be accomplished by an amount of fat possessing double the caloric value (26) the difference between the protein "sparing action" of fat and carbohydrate is not completely understood as yet.

Specific Dynamic Action:

Dynamic action may be defined as the energy expended by a subject in the utilization of food; by common usage the rate of metabolism of a subject under conditions of rest and in the postabsorptive state is spoken of as "basal". Heat

production is at a minimum. However, merely by the ingestion of food and with no accompanying physical exertion, the heat production of the subject rises above the basal level in amounts up to 30%. This phenomenon was already known to Lavoisier (27). There is a dynamic action involved in the metabolism of all nutrients, whether carbohydrate, fat or protein. The exact chemical reactions in metabolism causing a heat measurement are not known in full.

Until now the prevailing belief has been that the relatively high specific dynamic action of protein dominates the heat production of a subject fed a mixed diet, but evidence has accumulated since 1935 (28) which refutes this prevailing belief. Forbes and Swift (29, 30) have shown that contrary to general belief, protein was not shown to dominate the dynamic effects of nutrient mixtures. Rather, lard was found to be more potent than either protein or carbohydrate. These studies by Forbes and associates add much to our concepts of energy metabolism. It is clear that the dynamic effect of diets are not the additive dynamic effects of their components. In fact, there is probably no one value for the dynamic effect of protein, fat and carbohydrate, the dynamic value of each depending on the amount of the other two being simultaneously metabolized.

Temperature:

The influence of environmental temperature upon the metabolism of an organism must be borne in mind in any metabolic studies. Goto (31) Terroine and Trautman (32) and Houssay and

Artund (33) have shown that above 28° or 30° C. the heat production of rats increases (adult male or female rats). Horst, Mendel and Benedict (34, 35) in a re-examination of this problem have shown that for the adult male rat the most ideal temperature for measuring the basal metabolism of rats is at 28° to 30° C. and that within these limits there is no appreciable variation in basal metabolism. Hence, this contributing factor in the control of urinary nitrogen level remains constant under these conditions.

In studies of the above authors the humidity of the room and the size of the containing metabolism cage were also shown to be contributing factors in the control of the basal metabolism of these animals.

The conclusions reached in these studies were as follows:

1) The metabolism of adult male rats increased, on the average, 7.3% per degree decrease in environmental temperature below 30° C. (or ° F).
2) At thermic neutrality the metabolism of female rats was lower than that of males, both at young and adult ages, and with both sexes the metabolism decreases with age.

Level of Urine Volume:

In view of the fact that the products of catabolism are excreted in aqueous solution in the urine, the levels of urinary nitrogen are reported to be affected by either "retention" or "diuresis".

Voit (36) observed that the copious drinking of water is usually associated with a small increase in nitrogen elimination. Lusk (37) and Peters and Van Slyke share this view (38). Marshall (39) reported that twenty fold increase in urinary volume induced by the ingestion of very large quantities of water, lead to a two-fold increase in urea elimination in the urine.

On the other hand MacKay and MacKay (40) have shown that water deprivation caused an increase of urea in the urine, especially when dehydration is produced by intravenous administration of concentrated sucrose solutions.

DISCUSSION OF THE DYNAMICS OF PROTEIN METABOLISM IN RELATION TO PROTEIN STORES.

One of the earliest statements put forth as to the nature of protein metabolism was that of Pfluger's (41) which is essentially a modification of an earlier one by Liebig (1) in which he believes that there is a decided difference between circulating protein and living protoplasm; the former being comparatively stable toward oxidizing agents, while the latter is in a very unstable equilibrium and is partially susceptible to oxidation and further, that all protein catabolized first becomes an integral part of living tissue and only as such undergoes oxidation, a process which is supposed to constitute the most fundamental chemical decomposition of protein catabolism.

Pfluger's attitude with respect to the above is not one of unqualified commitment as in the latter part of the paper he practically admits the possibility of a certain amount being catabolized in solution, i.e., without having acquired the structure of living protoplasm. A view more in keeping with that of Voit (42) which was first formulated in 1867, and holds that the protein of the absorbed food passes through the blood to the different tissues and cells and is there catabolized under the influence of living protoplasm, but without first becoming an integral part of the latter.

O. Folin (20) in a presentation of a new theory of protein metabolism, interpreted Voit's statement as meaning that "living protoplasm is in a state of suspension, the circulating protein is in solution, and the chemical decompositions that constitute protein catabolism take place only in solution.

The small amount of living protoplasm which dies in the course of 24 hours is at first only dissolved, thereby becoming a part of the circulating protein derived directly from the food".

In 1905, Folin (20) after an extensive study of various urinary components under diverse nutritional conditions postulated his view of endogenous and exogenous protein metabolism. He stated that there were two forms of protein catabolism which were essentially independent and different, one being extremely variable in quantity and the other being constant. The former yielding in the urine, chiefly urea and inorganic sulphates, no creatinine and no neutral sulphur. The latter, constant, is largely represented by creatinine and neutral sulphur, and to a less extent by uric acid and ethereal sulphates.

And therefore according to Folin's view, protein synthesis in the adult organism, in nitrogen equilibrium, is restricted to the replacement of an increasing "wear and tear" or endogenous quota. This quota corresponds approximately to the minimum excretion of nitrogen, on a diet containing little or no nitrogen but otherwise adequate. When more than this minimal quantity of protein nitrogen is ingested, the excess over the endogenous requirement is considered to be quickly catabolized and appears in the urine, mainly as urea.

Some years later, experimental data began to appear, which contradicted Folin's, now classical, theories regarding protein metabolism. In 1913, McCollum and Hoagland (43) by

feeding CHO and alkaline salt, nitrogen-free diets to pigs increased the total endogenous nitrogen output in their urine without increasing the creatinine fraction, and likewise Teroinne, Bonnet, Danmanville and Mourot (44) argued that when the rate of endogenous nitrogen metabolism is increased there should occur a corresponding increase in creatinine and neutral sulphur. But it was found that the endogenous nitrogen metabolism, and later by Deuel, Sandiford and Boothby (45) that the basal metabolism can be increased without changing the excretion of creatinine.

Folin's concept of protein metabolism was found lacking in the development of other aspects of protein metabolism. McCollum (46,47), Osborne and Mendel (48) and Mitchell, Nevens and Kendall (49) all questioned whether the "wear and tear" quota really involved the destruction of any intracellular protein. The function of the "endogenous metabolism" according to these authors is to supply compounds such as essential amino acids for specific and indispensable but non-protein use. When these are supplied from the diet there is no need for the nreakdown of the tissues.

A complete break from Folin's classic concept was made in 1935 when Borsook and Keighley (50) proposed a new theory of protein metabolism completely difrering from Folin's view. They states that in an animal in nitrogen equilibrium the breakdown of intracellular protein is continually in progress even when abundant quantities of amino acids are obtainable

from the diet. This breakdown bears no "wear and tear" connotation, it greatly exceeds Folin's endogenous quota and is directly proportional to the level at which the nitrogen balance has been set at by the previous dietary history and therefore, as a consequence, in nitrogen balance, a corresponding quantity of amino acids is synthesized into tissue protein and peptides. When protein is ingested, in the course of the next 24 hour period, some of amino acids contribute toward maintaining constant concentration of free amino acids and peptides in the blood while the remainder is catabolized; this latter nitrogen appears in the urine. The last moiety might be called the "exogenous quota" distinguishing it from the urinary nitrogen arising from the catabolism of tissue protein and amino acids which is the "continuing metabolism" nitrogen of the above authors.

This new theory was based on two lines of experimental evidence, Experiments in which nitrogen balance was maintained for one day with non-sulphur-containing amino acids or ammonia, the sulphur was considerably in excess of the so-called "endogenous" sulphur excretion. In view of the fact that nitrogen balance was maintained, an increased "endogenous metabolism" could not be invoked to account for a sulphur excretion in excess of endogenous levels.

Other experiments indicated that, over one day periods, whether the body nitrogen is spared by a protein or by non-sulphur-containing amino acids or ammonia, the same

amounts of sulphur and, by inference, of nitrogen are contributed to the urine by tissue sources. These contributions from the body could not have come from the pool of circulating free amino acids and must have come from protein and peptides because concentration of the former in the tissues is remarkably constant and independent of the dietary state and remains unchanged even in starvation (49,51,52,53,54).

Even before isotopes were available for tissue studies it was necessary to envisage extensive synthetic processes continually in operation and greatly in excess of those required to replace the losses of the "wear and tear" quota of the classical theory. Madden and Whipple (55), from their experiments on plasmapheresis and replenishment of plasma proteins, conclude that a dynamic equilibrium exists in the body whereby proteins of the plasma, liver, and other tissues are constantly exchanging.

Schoenheimer and collaborators (18) using N^{15} as a tracer, furnished direct evidence that nearly all proteins of the body are continually undergoing synthesis and breakdown. In the liver and intestinal mucosa, more than half of the protein is broken down and resynthesized in ten days; in the muscles it is slower, and still slower in the erythrocytes.

Schoenheimer et al (66) using glycine, l-leucine, d-leucine, and dl-tyrosine labelled with N^{15}, found that the rate of incorporation of dietary isotopic nitrogen in plasma proteins was approximately the same as in the proteins of the kidney, liver and intestinal tract. All fractions of the plasma, fibrinogen, euglobulin, pseudoglobulin and albumin, interchanged N^{15} at the

same rate. In contrast to the plasma proteins, porphyrin and the proteins of erythrocytes exchanged N^{15} very slowly, indicating that the cycle of degradation and synthesis of Hb is much slower than in organ and muscle proteins.

Tarver and Schmidt (57) using radioactive S as a tracer, obtained results similar to Schoenheimer and reached the same conclusion, viz., a continual synthesis and breakdown of body protein, the rate varying in different organs and with different proteins.

Mitchell, Burroughs and Burroughs (58) in criticizing Schoenheimer's conclusions, maintained that "In all likelihood, the chemical reactions that Schoenheimer has detected by means of isotope nitrogen, between dietary amino acids and tissue protein, relates not to the fixed protein of cells, but to the dispensable reserve proteins, readily subject to mobilization by many experimental procedures and is readily reformed".

However, Tarver and Schmidt (57) showed that no separation can be made between fixed and "reserve", that is labile, tissue protein. They fed methionine containing radioactive sulphur to rats fasted 3 to 8 days, and to dogs fasted 8 days and found radioactive sulphur in the proteins of many organs.

The last remaining support of the classical theory was removed when Bloch, Schoenheimer and Rittenberg (58A) found that urinary creatinine is derived only from creatine in the tissue and that creatinine once formed is not degraded further and urinary creatinine remains undiminished even on a protein-free diet.

As was predicted from the new theory, the protein sparing action of CHO occurs in the well nourished animal as well as in the starving animal and was verified experimentally in the dog by Larson and Chaikoff (59) and in man by Cuthbertson et al (60). Larson and Chaikoff observed in dogs, in nitrogen equilibrium, a retention of nitrogen when extra glucose was given at ot nearly at the same time as the protein, and when extra glucose was withdrawn, the stored nitrogen was slowly excreted. According to the classical theory (Folin's), no storage was to be expected because in well fed animals in nitrogen equilibrium, the "wear and tear" quota is supplied by the diet, and the remainder of the dietary nitrogen is the exogenous and should be quickly catabolized.

In summation:- the growing body of experimental evidence points to a dynamic concept of protein metabolism where even in the fasted animal there is an active and simultaneous breakdown and resynthesis of protein.

To introduce the concept of "labile" or "reserve" protein at this point might seem unwarranted, as there is an implication in the terms "labile" and "reserve" that this protein is somehow different from the main mass of body protein, a point seemingly in contradiction with the results obtained by labelling S and N, although a vast amount of evidence, appears in the literature to support the concept of reserve protein store.

PROTEIN STORES

The reserve store of protein may be defined in the manner of Madden and Whipple (55) "As all protein which may be given up by an organ or tissue under uniform conditions without interfering with organ or body function". (No difference physiologically or anatomically from rest of body proteins).

Many investigators believe that to justify the term the stored protein should exist as a physically demonstrable entity comparable to glycogen and fat intracellular masses, and some claim to have found such protein deposits (61,62), these observations remaining, as yet, unconfirmed. The careful analyses of Luck (63) of the protein of the rat liver after storage has been induced, indicated all fractions of this protein to have participated equally in this storage process.

Early evidence for the existence of such a store of reserve protein is seen most clearly in the mathematical treatment of the lag in attainment of nitrogen balance in passing from one level of nitrogen intake to another. Martin and Robinson (64) discovered the relation that the utilization of the stored nitrogen follows the course of a first order reaction; only the "labile nitrogen" undergoing this rate of catabolism because this first order degradation stops abruptly long before any significant loss of body protein occurs (45). Other early evidence of a storage of "labile nitrogen" is given by Chambers and Milhorat (65) in which they show that in normal exercised dogs the extra urinary nitrogen appearing from work can be completely spared by carbohydrates. It gradually disappears as the fast of the animal is continued.

Cuthbertson et al (60) showed that in adults, the superimposition of various proteins (for one day) on a basal diet, which had previously been shown to suffice for the maintenance of nitrogen equilibrium, caused a retention of nitrogen and sulphur. The degree of retention of nitrogen and sulphur bore a quantitative relationship to the total increment in the energy value of the diet, the retention of the nitrogenous material diminishing with time. On resumption of basal conditions the retained nitrogen was eliminated at a slow even rate.

That the nitrogenous metabolites are stored as protein appears probable from the weight of evidence reviewed by Borsook and Keighly (50). These investigators present new data to indicate that in an adult man in nitrogen equilibrium and a urinary nitrogen excretion of about 10 grams daily, about half of this nitrogen comes from catabolism of stored protein, the extent of which storage is a function of the previous dietary history, this fraction being termed the "continuing"nitrogen metabolism. In this dynamic picture, extensive synthetic processes involving amino acids balance the catabolic portion of the "continuing" nitrogen metabolism.

That general protein body "stores" might exist is indicated in the work of Addis, Poo and Lew (66,67) who measured the decrease in the protein content of various organs in rat fasted over a period of days. They observed that the proportions of the original protein content lost by various organs was widely different, the liver losing 40%, prostate and seminal vesicles 29%, heart,

kidneys, drawn blood and alimentary tract 18-28%, muscle skin and skeleton 7% and bone 5% of its protein content respectively, and also approach the problem from the opposite viewpoint, that is, the superimposition of protein in various organs on different dietary protein levels, and found that each level of protein intake was associated with its own characteristic pattern distribution of protein amongst various organs. On diets containing 6, 11, 16, 27, and 43% protein, the greatest total gain in body protein occurred surprisingly enough, on diet containing only 27% protein. Each organ and tissue had its own mode of reaction to an increasing supply of food protein.

Luck (68) has also shown that the administration of high protein diet to various animals resulted in enlargement of the liver and an even greater increase in its protein content, and brings forward two possible interpretations of this observation:

"the liver can be said to convert the rich influx of amino acids into "reserve" protein which is then deposited in the organs as (inert storage material) or, alternately, it might be maintained that the protein so deposited, far from being inert storage material, represents an increase in functioning hepatic material" - a view more in keeping with the observations of Schonheimer (18) and Tarver and Schmidt (57). Whether the increase in liver cells occurs to handle the increased work of deamination and urea formation (68) to which it is now subject

remains problematical, although it has been shown that during fasting the liver is one of the first organs which most readily sacrifices its protein content.(66).

That the liver has no "storage" protein which is distinctive in its physiologic properties and chemical differentiation is supported by the following observations:

(a) Grund (69) did not observe any change in P/N ratio of liver in fasting.

(b) Lee and Lewis (70) failed to find any significant change in the relative amounts of cystine, tryptophane and tyrosine in liver, kidney or muscle protein with change in nutritional state of the animal and more recently Kosterlitz and Cramb (71) found no significant change in either the $\frac{\text{protein nitrogen in liver}}{\text{phospholipid phosphorus}}$ or $\frac{\text{muscle protein nitrogen}}{\text{muscle protein phosphorus}}$ of rats fasted for 2 days.

(c) Luck (63) found that the various fractions of the liver proteins increased equiproportionally with increased protein intake and none of the liver proteins could be singled out as a reserve, labile or cell inclusion protein in the classical sense.

(d) And last but not least, Tarver and Schmidt (57) working with radioactive sulphur showed that no distinction could be made between reserve store (i.e., more labile in response to fasting, damage, etc.) or tissue proteins. In the feeding of methionine labelled with S^x to rat fasted 3-8 days and dogs fasted 8 days, it was found that S^x was taken up into the proteins of many organs in spite of the fast.

A fascinating experimental approach to this problem is the one employed by Whipple and co-workers (72) - namely, "plasmapheresis". From their experiments (to be discussed below) they conclude "That a reserve store of plasma protein building material is one subject for which the evidence is overwhelming and it may be accepted as a fact".

That such a store exists was first demonstrated by Morawitz (72A) in 1906, when he found that regeneration of the blood plasma proteins occurred during fasting following their acute depletion by bleeding. He combatted anaemia by injections, simultaneous with the bleeding, of an equal quantity of red blood cells suspended in Locke's solution to which 3% gum acacia had been added. This was the first use of a technique, later called "plasmapheresis" by Abel, Rowntree and Turner (73).

Morawitz found that by a large one-stage plasmapheresis the total plasma proteins of a dog could be reduced to about 2% and that return to a normal level occurred during fasting, in 2 days in one instance and in 4 days in another. In the second instance, the dog was depleted again at 4 days, this time the reduction of the total plasma concentration to 3%, and regeneration was observed, reaching a level of 5.4% in 3 days, (the normal being 6-7%) all in the fasting animal.

By another method, Whipple et al (74,75,76,77) have measured what they term "the reserve store of plasma protein building material" - one that may not be synonymous with the labile store that Cuthbertson (78) claims.

The method and the calculation determining this store have been described in detail by Madden et al (72) but a brief summary will suffice here. The normal dog is depleted of circulating plasma protein by daily plasmapheresis while consuming a constant basal diet low but adequate in protein. With the normal dog it will be found necessary to remove more plasma (and plasma protein) in the initial days or weeks of the regime than in subsequent weeks in order to maintain a steady hypoproteinemia. This "excess quantity" of plasma protein removed in the first weeks of a prolonged period of plasmapheresis represents the "reserve store". It is a greater quantity of protein than is represented by the difference in the circulating mass of plasma protein before and during experiment, (i.e., 6%-4% x plasma volume equals about 9 grams plasma protein in a 10 kg. dog), its measurement being graphically represented in Fig. 1.

Table taken from Madden and Whipple

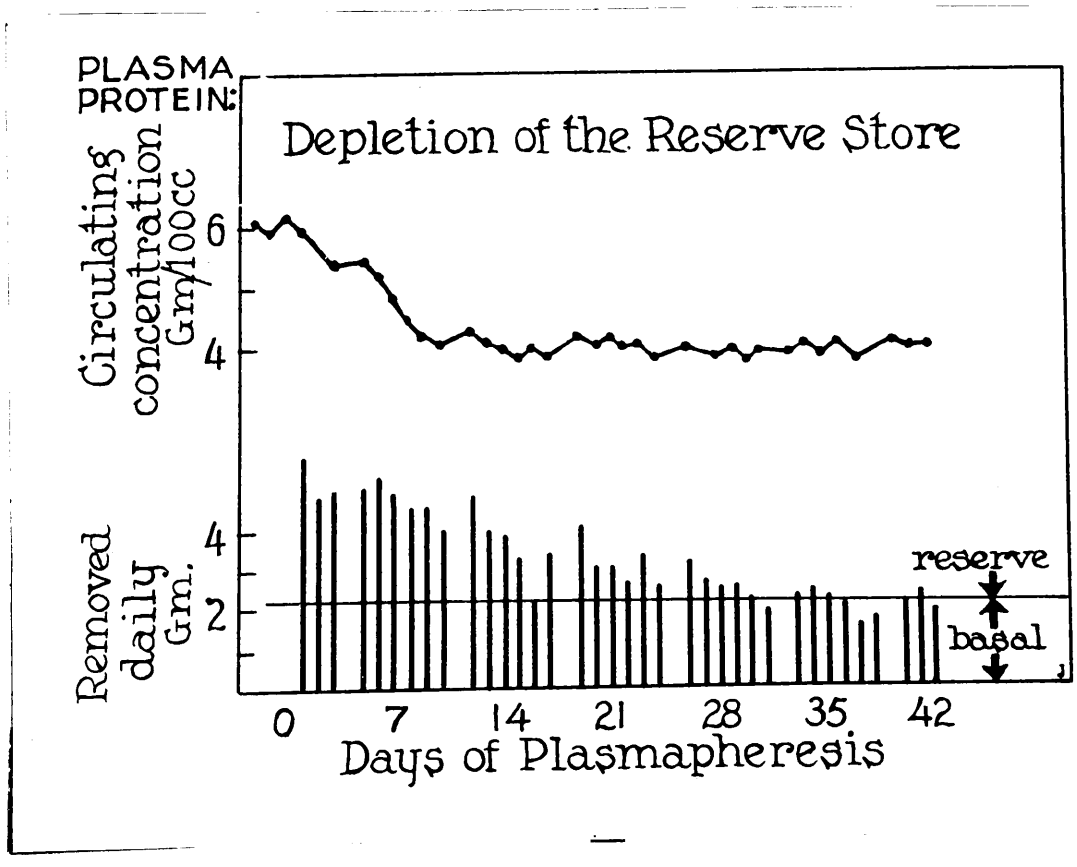

Fig. 1

The excess production noted in the initial 4 weeks (Fig.1), must be attributable to protein materials present in the body at the start of the experiment.

Fasting experiments have yielded valuable results and have been carefully studied in Whipple's laboratory. Given a standard anaemic dog fed an optimal oral dose of iron with sugar and fat but no protein, they obtain 40-50 grams new haemoglobin each week for several weeks. Obviously the protein (globin) must be formed to a considerable degree from body stores presumably held in cells in some protein form. Such dogs show less nitrogen in the urine especially in the urea-ammonia fraction as compared with normal non-anaemic controls. This might indicate that the precursors of urea were utilized to some extent in the building of new haemaglobin under these conditions (i.e., a shift of body protein or breakdown products thereof) somewhat reminiscent of Miescher's (79) observations with salamanders, where he observed that the influence of diet was only of secondary importance in the restitution of amputated limbs in these animals receiving no food, regenerated their limbs as rapidly as well fed animals.

Howes (80) found that rate of healing of stomach wounds of adult rats was not noticeably affected by complete starvation or half feeding, so that in both cases one might infer that the animals have drawn upon "readily available stores" for the reparative processes.

From the experiments of Whipple et al, it may be estimated that normal dogs have different materials in storage to form a quantity of plasma protein one to two times that normally present in their circulation. This may amount to as much or more than the total protein content of the liver and indicates that a large portion must be stored elsewhere, being larger than that given by plasma protein regeneration during fasting (72,81,82) and probably more nearly represents what the animal can produce when the only demand on the stores is for plasma regeneration. Dogs once subjected to plasma depletion and thereafter allowed to return to normal, exhibit larger reserve stores on subsequent depletion (72).

Other experiments performed in Whipple's laboratory reveal the process of "storage of plasma protein" forming materials. Such stores can be experimentally induced and reduced quantitatively.

In a standardized protein depleted dog, plasmapheresis was discontinued for 2 weeks and materials which ordinarily would have been removed as plasma protein were stored in the body depots, to be removed quantitatively as new plasma protein during the subsequent three weeks of plasmapheresis.

A further example of the ability of the body to retain plasma protein forming materials in time of need is given by observations in a previously depleted dog that ate over 400 grams of raw pork kidney in one brief meal (75). The dog

conserved the nitrogenous constituents of this single meal as efficiently as it subsequently did a somewhat similar quantity consumed over a period of 7 days.

Using somewhat similar techniques Melwick, Cowgill and Burrack (83), have found that when dogs are fed a protein-free, adequate vitamin and caloric diet and when simultaneously subjected to quantitative plasmapheresis, whereby a basal level of serum protein concentration was reached and maintained constant, then the dog is able in one week (with the above dietary) to regenerate 20 to 30% of the total amount of the blood protein normally present in the plasma, which they infer is the "reserve store of serum protein building materials normally present in the circulation".

These "reserve stores" can be replenished and the animal kept in nitrogen equilibrium not only by the administration of an adequate diet but also by the intravenous injection of homologous plasma (74). Also during a fast a dog produced 30-50 grams of new haemoglobin within a week and another dog, while fasting, was able to regenerate new liver cells in repairing extensive hepatic injury due to chloroform poisoning (84).

It is also interesting to note at this point that plasma can contribute to the body's needs and may be considered in dynamic equilibrium with body tissue protein as has been noted by Whipple et al, that various dogs could be kept in nitrogen and weight equilibrium by intravenous plasma injections with oral fat and carbohydrate supplements (77,85,74).

This view is in keeping with that of Schoenheimer (56) who, using glycine, l-leucine, dl-leucine and dl-tyrosine all labelled with N^{15}, found that the rate of incorporation of dietary isotope N^{15} into plasma proteins was approximately the same as that in the proteins of the kidney, liver and intestinal tract and also that all the fractions of the plasma proteins, viz., fibrinogen, euglobulin, pseudoglobulin and albumin interchanged N^{15} at the same rate.

From the above experimental observations Madden and Whipple (55) conclude "a part of the body protein forms a reserve against adversity in the sense that it can be circumspectly depleted without apparent injury to the body. The supplies of amino acids coming from outside the body, and the demand for protein material within the body are in a constant state of balance with these protein "reserve stores", a dynamic equilibrium".

This last, probably being by far the most important concept which derives from all these experiments, i.e., the fluidity of the body protein (including plasma protein) a ready give and take between the protein depot - a dynamic equilibrium (86).

Borsook and Dubnoff (87) Schoenheimer and Ratner (88) have independently suggested, and a reconciliation between the two may be in that the "labile" nitrogen is not distinguished primarily by a difference in composition, but by its location, as many attempts have been made to distinguish this "labile" protein from "fixed" organ protein. All these attempts have failed (63,70,89,90). Some organs, the liver, intestine, and kidney, change in size and protein content quickly with changes

in level of dietary protein. The "labile" protein will then arise in the course of a fast, at first from the substance of the labile organs. But these organs can increase or decrease only within certain limits. Then, as the fast is prolonged and the lower limits of size of the more labile organs are approached, larger amounts of nitrogen will come from the organs slower to change, such as the muscles. The liberated muscle nitrogen will, in part, be resynthesized into liver and kidney protein; the remainder will participate in maintaining the characteristic and constant concentration of free amino acids in the blood and tissues and, in so doing eventually be catabolized.

Thus, in the course of a fast, the source of the so called "reserve" protein will shift from organs which change rapidly to those which change slowly, and hence its composition and the N/S ratio in the urine will change. This explanation is in accord with the failure to indetify any definitive "reserve" protein with the changing N/S ratio of stored nitrogen and urinary nitrogen in the course of a fast (45,91,92,93), with the variations in protein content of different organs in the course of a fast and on subsequent feeding (64,94,95), and with different rates of interchange of isotopes of different organs (18,57).

One is tempted at this point, to ask then what is the determining factor which marks off body protein from "reserve" protein. In the opinion of the writer the only criteria that

may be adopted in the tentative classification of the above two, is that the latter is immediately utilizable and the former being less readily available in any emergency - fasting, post-damage, etc. As Youmans (96) emphasizes "recent studies with isotopes indicate that there is a general pool of nitrogen formed from dietary and reactive body tissue nitrogen from which protein may be formed for any purpose. With more severe deficiency the tissues of the body are drawn on for protein to supply the most necessary purposes, the less important structures being drawn upon first. In severe deficiency even such important organs as the heart yields some of its protein. The protein of the active parenchynal cells of such organs as the liver seems to be affected last".

If one agrees to the above mentioned classification, then it may be noted that in the utilization of the "reserve store" for general nitrogen requirements or for plasma protein formation, it is characteristic that a large bulk of the store is used early and rapidly.

(1) Addis, Poo and Lew (66) found that half of the total protein of the liver to be lost during a 7 day fast, is lost during the first two days. Considering that 40% of the protein content of the liver of a rat is lost during a 7 day fast (67), liver storage protein appears readily available. Its rate of conversion is probably no greater, however, than that of the greater mass of stored protein found in the carcass of the rat. This (last) mass lost four times as much protein

as did the liver and from the data given it was available at the same rate, 50% within the first two days. Its rate of conversion is probably no greater, however, than that of the greater mass of stored protein found in the carcass of the rat.

(2) Following rapid severe depletion of plasma proteins in the experiments discussed above (72,82) the rate of regeneration from stored protein was most rapid in the first few hours.

(3) In the depletion of the reserve stores (72) the larger proportion is removed during the initial week or two, but similar quantities may continue to be contributed by them for as long as five or six weeks.

An explanation for the above is contained in the proposal advanced by Madden and Whipple (55) "When the steady state existing between body protein supply and demand is disturbed the rate of reaction in direction of a new equilibrium, is determined by the product of the active masses of the substances reacting as well as by other constants and conditions".

Thus, when the mass of stored protein is large, and change of diet, plasma depletion, body injury, or other disturbing factor is introduced, this mass will be converted at a faster rate than when the mass is diminished, other things being equal. Therefore, under such a concept the "labile" portion is merely that portion of the "reserve store" which is mobilized first and fastest and in other respects is not different from that made available more slowly.

A curious fact which bears upon the plasma formation is the remarkable increase in blood cholesterol which is observed during the hypoalbuminemia of nephrosis and is also alleged to occur during experimental hypoproteinemia (97). Its significance is, as yet, not clear.

Another experimental approach, although more direct, in favor of the existence of a readily available protein "reserve" is that of MacKay et al, (98). He and co-workers have shown that the fasting ketosis of rats is more severe, the lower the protein content of the preceding diet, apparently because the prior protein intake determines the amount of reserve protein available for catabolism during fasting. That this reserve store is a source of anti-ketogenic material is indicated by the fact that fasting rats, previously fed a high protein diet, better maintain their glycogen and blood sugar, as well as showing lower levels of ketonemia, than rats on a prior low protein intake.

Worthy of mention at this point are experiments independently conducted in the laboratories of Weech (99) and Elman (100). Both observed a pronounced fall in the concentration of the albumin fraction of the plasma in dogs fed a protein deficient diet for three weeks and a similar but smaller fall occurred in the albumin fraction of dogs fasted completely, the globulin fraction remaining unchanged. A return to adequate feeding is followed by albumin regeneration. The less pronounced hypoalbuminemia in the animals completely

fasted was accompanied by an increased blood cell volume and was therefore, due in part to dehydration. If there is such a thing as deposition or reserve protein, it certainly fails to manifest itself under the conditions of these experiments.

Melnickm Cowgill and Burrack (83) reported a dog which was subjected to plasmapheresis and low protein diet. This was carried for a period of 50 days until a steady low level of hypoproteinemia was reached and maintained. Then on subsequent fasting it was noted that after an initial delay a progressive regeneration of serum protein took place. This increase in serum protein was actually due to regeneration of the blood protein and not to hemoconcentration as evidenced by relative plasma volumes. If therefore, one assumes a steady low level of hypoproteinemia (ca. 4 grams %) as indicative of depleted protein stores as do Madden and Whipple (55) then in the above example the animal is drawing upon sources other than these during the fast.

Turning our attention to the effect of low or protein-free diets on urinary nitrogen excretion in rats and dogs, the recent report of Cuthbertson and Munro (78) is of interest. They fed rats a protein-free diet until a steady low level of nitrogen output was maintained. The left femur of these rats was then fractured, and these rats, still fed the same diet, failed to show a rise in urinary nitrogen output as seen in rats previously fed different levels of protein intake.

They concluded, therefore, that the excessive output

of urinary nitrogen, following injury (in rats fed diets containing adequate protein) arises from a readily exhaustible labile protein reserve.

Other similar observations have been made by various authors (101,75,102). A dog with long standing anaemia (101), whose so-called reserve store was considered depleted by frequent bleeding until a steady low level of haemoglobin concentration had been reached, on being subjected to a sterile abscess, gave a clinical picture similar to that in the non-anaemic dog - i.e., fever, leucocytosis, pus formation, etc., but the elimination of urinary notrogen was not increased in the anaemic dog as was in the normal one in response to abscess formation. The same was observed in dogs made hypo-proteinemic by plasmapheresis (75).

In the period immediately following trauma (fracture, abscess formation) the absence of increased urinary nitrogen levels (which is the observed response of a previously healthy individual) may be due to:

(1) Depletion of stores, which Cuthbertson (78) and Madden and Clay (103) maintain is the source of extra urinary nitrogen excreted after damage in the previously healthy organism.

(2) The low or absence of increased nitrogen output of the "depleted animal" may be due to conservation of the metabolic precursors by their recapture. Following on this one would expect that only in the face of conditions which

are a direct threat to the animal (depleted) will it draw upon nitrogen metabolites.

(3) It may also be that the metabolic requirements of the previously damaged organism's metabolic requirements are very high, whereas those of the "depleted" animal are much lower in this state, as it has been shown that persistent feeding of low protein diets leads to decreased metabolic requirements (35).

Or a combination of (2) and (3) may account for Cuthbertson's observations (78).

The evidence for (3) lies both in the writer's observations (under experimental section) and in an observation of Weech, Goettsch and Reeves (104). A dog was maintained on a protein-free, otherwise adequate diet until both the serum albumin and total plasma had reached a steady low level (globulin remaining fairly constant). Also the nitrogen excretion had likewise reached a steady low level. On the 65th day of this regime a self imposed fast began which lasted for ten succeeding days, except for salt and water given by gavage. This anorexia was not associated with fever or infection and during this period of fasting the urine nitrogen rose from a previous steady low level to much higher with considerable loss of nitrogen. The serum protein and albumin rose to within normal values during this period with no evidence of hemoconcentration.

Here, therefore, is a dog whose reserve stores were supposedly depleted, regenerates, during a fast, serum protein and catabolizes tissue protein with consequent increase in urinary nitrogen. In subsequent course of events when the

dog began to eat voluntarily the serum protein and albumin gradually declined as during the early days of the experiments.

It would, therefore, appear that when a "depleted" animal is subjected to an injury which does not threaten its immediate existence, it conserves those nitrogenous metabolites which are drawn upon to meet its metabolic requirements during fasting.

(4) It might be that in the "depleted" organism a lack of response on the part of the adrenal cortex to put out increased amounts of gluconeogenic substances after damage might explain in part the failure to observe a rise in urinary nitrogen during this period, either due to atrophy of the cortex, per se, or failure of the pituitary to secrete increased amounts of corticotrophin after damage.

Curious is the observation that only a diet of raw carrot or carrot powder and water is productive of a steady and maintained hypoproteinemia in rats (105,106). An otherwise low or protein-free diet is however without effect on the plasma protein content in rats.

In the experiments of Cuthbertson the rats were damaged on the 14th day of feeding of the protein diet. In the work of other investigators it required from 16-18 weeks to reach and maintain a steady hypoproteinemia. Therefore, if one accepts hypoproteinemia as the criterion of depleted protein reserve rather than a maintained low urinary nitrogen output, the rats of Cuthbertson's should theoretically still

possess a considerable protein reserve.

In view of the fact that hypoproteinemia due to deficiencies in dietary protein cannot be prevented by body tissue protein or some other source of endogenous protein, the concept of reserve protein may perhaps not apply to the lack of an observed catabolic process occurring after damage in "depleted" animals.

SOME PHYSIOLOGICAL ASPECTS OF POST TRAUMATIC METABOLIC RESPONSE.

The metabolic response to injury, being a complex one, is arbitrarily divided into several phases. First, the loss of tissue substance and varying degrees of cellular damage at the site of injury. This represents an immediate loss of protein and other cellular and intracellular substances. The metabolic activity of neighbouring cells is disturbed and therefore need arises for amino acids to replace or heal the injured tissue, which will vary according to the nature of the tissue lost or destroyed and the degree of fibrous repair.

Usually there is immediate and well marked disturbance of cellular vitality in the injured zone due to the damage done. The capillaries dilate and their permeability increases, leading to what has been called "reactionary oedema" and to temporary depression of local metabolism. The effects of the lesion spread and there is a discharge of leucocytes from bone marrow.

Providing damage done is not fatal and depending on the nature and severity of the wound and the loss of blood or plasma externally or into the tissue spaces, there will be a period of reduced blood volume and "oligaemic shock" may develop. This phase of post injury metabolism is characterized by a definite metabolic depression during which the blood flow to the kidney, liver, etc., is cut down to preserve the circulation of the more vital centers and the function of these organs suffers in consequence.

That a general depression of metabolism occurs during this period of traumatic shock has long been recognized

Declasse (107) in 1854 and Meltzer (108) in 1908, and has been shown by Henderson, Prince and Haggard (109) in 1917, in burns, to amount to a decrease in oxygen consumption of 45-50% in the cases studied.

Davis (110) and others have found a reduced oxygen consumption in shocked dogs. Aub, 1920, (111,112) found that the degree of fall in the basal metabolism of cats was roughly proportional to the severity of the shock produced and that recovery after transfusion was usually associated with a prompt return of the metabolic rate to normal.

The above changes during this period of depressed metabolism are accompanied or characterized by certain changes in the biochemistry of the blood which vary according to stage of depressed vitality or compensatory reaction reached. There is evidence of profound cellular disturbance, which in later stages may be characterized by a large increase of potassium in the blood and tissue fluids, Scudder (113) and also an increase in blood creatine may occur. And as shock developd, other changes have been observed, such as hyperglycemia and some degree of nitrogen retention in the blood. In the above state the body temperature is in general lowered despite the diminished peripheral circulation. Of all changes produced, the tissue anoxia is probably the most dangerous.

Following this period of shock with lowering metabolic activity and with return of urinary flow to normal, there sometimes appears in the urine the products of damaged tissues;

this is mainly found in severe injuries such as crushing injuries, Bywaters (114). There is later a rise in metabolic activity which has been termed "traumatic inflammation" or the period of "traumatic nitrogen deficit" (115) or "protein catabolic period" (116), as it is mainly characterized by the high levels of urinary nitrogen and a period of negative nitrogen balance.

Then pioneering work of Cuthbertson starting in 1929 (117) from studies on the calcium and phosphorous metabolism in bone fracture cases brought to light certain outstanding metabolic features occurring in the post-traumatic period. Whereas the intake and output of calcium showed only minor discrepancies, there was a definite loss of phosphorous from the body. Further studies revealed a well marked loss of nitrogen, sulphur and phosphorous in above cases during the period of rising metabolic activity in the post-traumatic phase. The rise in the excretion of nitrogen and sulphur being due to increases in the urea and inorganic sulphur output and there was an accompanying creatinuria. The same occurred on constant dietary intake and parallel with loss in nitrogen a rise in oxygen consumption occurred, although their peaks often did not coincide.

That disuse atrophy, due to immobilization of limb by splinting, only contributed in small part to above was shown on normal controls (118), where only a small rise in nitrogen, sulphur and phosphorous, and to a lesser degree of calcium was shown. All these changes being slight compared to those noted in the fracture case studies.

The loss of body substance represented by the urinary nitrogen and sulphur was not derived solely from the area of injury as dislocation of ankle (119) produces as large a metabolic disturbance as splintering of bones, and in the rat (120) this general wastage of body substance cannot be fully accounted for by the loss of muscle substance from the site of injury or from injured limbs. The conclusion has been reached by Cuthbertson (120)"that there would appear in addition, a generalized increase in catabolism to meet the exigencies of the enhanced metabolism of the recuperative process".

Earlier investigators had encountered this extravagant post-traumatic nitrogen loss. Malcolm (121) who noted evidence of an increased nitrogen metabolism following a major operation although the temperature was low. Hawk and Gies in 1904 (122) confirmed observations of Bauer (123) and also those of Jurgensen (124) that haemorrhage caused an increased elimination of urinary nitrogen, and showed that even venesection, without blood letting is sufficient to cause an appreciable though slight increase in urinary nitrogen and sulphur in the dog. Werthermer, Fabre and Clogue (125) noted that after a period of shock, the amount of ammonia and urea excreted, remained at a high level for some time and might even amount to 37 mgms. urea per day.

That this phenomenon, i.e., excessive loss of nitrogen and sulphur, is ascribable to any insult the organism might suffer is borne out in observations of early observers that during infectious diseases, a period of negative nitrogen balance occurs which could be alleviated but not prevented by large excesses of dietary carbohydrate and fat (126).

Excessive losses of urinary nitrogen have been reported in a variety of other pathologic conditions; as after acute haemorrhage (127,128), operations of all sorts and injuries, mild and severe (119,129,130,131) and in gastrointestinal obstruction (132,133,134,135,136,137).

In some cases these losses may be attributed largely to starvation and inadequate feeding and in others to exudation of protein containing fluid, but ruling these out, the phenomena of an ensuing negative nitrogen balance following trauma can now be considered an established fact, as diets rich in first class protein, and containing the maximum of calories which the patient could consume, failed to eliminate the negative nitrogen balance at the height of the catabolic process in many cases of fractures.

In an effort to elucidate the underlying mechanism involved in the catabolic period which results in heightened nitrogen excretion, various experimental approaches were employed. As has been stated, Cuthbertson showed that wasting through disuse atrophy offers but a partial explanation for the nitrogen loss. Some reflex trophic effect may possibly be in operation, such as is found in the muscular wasting associated with bone or joint disease. Harding (262) has shown that such an atrophy, which is mainly reflex in character, is accompanied by an increased consumption of oxygen. Such a theory demands an exhaustion of the muscle by an excessive number of abnormal stimuli. This reflex wasting of muscle is undoubtedly responsible, in part, for the increased tissue catabolism which has

been observed during the convalescence of patients who had their knee joints incised. It is questionable, however, if this type of "sympathetic" wasting is the main cause of the greatly increased protein catabolism after injury.

Reflex wasting would presumably apply solely to the limb affected and not to the body generally, a view not in keeping with the results of metabolic studies on rats who have had either one of their femurs damaged, in which Cuthbertson found (120), "In previously healthy rats the metabolism of nitrogen, phosphorous, sodium,creatine and creatinine were studied, as well as the change in weight of the muscle groups affected, after the fracture of the femur by open operation and without splinting. It was found that the osteotomy was followed by a well marked disturbance of general as well as local metabolism, and this was again evidenced by a well marked loss of nitrogen, phosphorous and potassium with some increased creatinuria. Sodium and creatinine remained relatively constant; occasionally a slight fall noted. These products as a rule reached their maximum elimination about the 3rd or 4th day in the rat, slightly earlier than they did in man, although the findings in man exhibited considerable variation. There was often a small rise in the excretion of these variable catabolites several days later. Following the peak of the metabolic disturbance, the various processes declined in intensity, paralleling the changes seen in man where there results a swing in the pendulum resulting in a marked positive nitrogen balance".

From weighing the limbs and muscle groups involved (120), it was obvious that the wastage of body substance could not fully be accounted for by the loss of muscle substance from the site of injury, or indeed even from the injured limb, and there appears to be in addition to regular wasting and autolysis, a generalized increase in catabolism.

EFFECT OF DIET AND OTHER FACTORS ON NITROGEN METABOLISM AFTER TRAUMA.

Morgan's (138) early experiments on salamanders demonstrated that the influence of diet on the regeneration of tissues can only be of secondary importance, for salamanders receiving no food regenerate their amputated limbs just as rapidly as do well fed animals. Howes and others (80) found, too, that the rate of healing of stomach wounds of adult rats was not noticeably affected by complete starvation or by a half adequate diet.

Evidence was obtained (120) that additional carbohydrate exercised a definite sparing effect on the general loss of tissue substance in damaged rats as evidenced by the urinary nitrogen, although it did not appreciably affect the local tissue wastage. In patients with fracture of femur or of the leg bones, given diets rich in protein, calories, or both, it was found that these diets considerably ameliorate the urinary losses (139). However, diets rich in first class protein and containing the maximum amount of calories which the patient could consume (as much as 230 grams protein were consumed daily by one patient and 4,100 calories by another) failed to eliminate completely a negative nitrogen balance at the height of the catabolic process. After raising the intake there occurred first a period of retention of nitrogen while the nitrogen excretion was rising, then a period of loss to be followed later by another period of retention as the high nitrogen excretion subsided with the passing off of the catabolic period. The failure to attain nitrogen equilibrium

was more than surprising, since there was little if any disturbance of body temperature and since it had been previously found that when excess food is given to a normal uninjured person a well marked retention of nitrogen and sulphur occurs temporarily (60), which was later slowly excreted whem returning to normal (maintenance) dietary regime. It may well be then that calories or protein, (or both) requirements are much higher in this period and that the required levels were not attained.

That this may be so is shown in a more recent paper by Taylor, Stevenson, Davidson, Browder and Lund (140) in which they produced evidence that large amounts of protein, up to about 290 grams per day would establish nitrogen balance very early after time of damage in burned patients and Croft and Peters (141) have shown in burned rats that many dietary protein levels from 10% casein to 18.2% casein substantially reduces loss of urine nitrogen as seen in balance method. That this may be confined to burns above is not yet proven, but one must bear in mind the concomitants of this form of damage which doubly necessitates high protein intake - loss of skin protein by sloughing off and loss of plasma-like fluid into burned areas.

METHIONINE.

The theory has been proposed that the period of increased metabolic activity which is seen after moderate or severe injury, might well be one of increased protein catabolism, involving the liberation of some essential key amino acid or

or acids for repair, and the oxidation of the others for energy purposes.

Croft and Peters (141) have shown in a recent paper that the urinary losses in nitrogen observed after burning were reduced substantially by the inclusion of 1% methionine in the diet. Neither alamine nor a mixture of synthetic amino acids nor cystine had a similar effect. It is suggested by them that the apparent toxaemia is in reality a deficiency, induced by a necessity for one key amino acid with consequent raiding of a whole protein molecule.

A study of two burned patients in this laboratory, however, (142) (where methionine supplements in greater proportion to body weight as compared to amounts used by Croft and Peters) failed to confirm the observations of Croft and Peters. Whether the metabolic pathways are different in the man and rat in this post-damaged state or whether other supplementary dietary factors are involved or not, is as yet, not clear.

METABOLIC FUNCTIONS OF THE ADRENAL CORTEX.

There is little doubt that the adrenal cortex plays an integral role in the resistance of an organism to stress. An extensive list can be constructed of drugs, poisons, toxins, infections, altered environmental conditions and, especially, traumatic procedures to which the adrenalectomized animal is highly susceptible. No attempt is made to discuss in detail all of the above, but, attention is focused on its function in traumatic injuries.

That the adrenal cortex is concerned in the ability of the normal animal to withstand traumatic injury is not unlikely, and has been postulated (142,143). The proof would be in the clear demonstration of a protective influence of cortical extract against the development of shock in the intact animal. Browne, Weil and Rose (144) and Selye et al (143,145) likewise reported that desoxycorticosterone was of no value in treating shock in rats and rabbits following intestinal trauma, but found that cortical extract or the corticosterones were useful.

Perhaps the best evidence in favor of a beneficial effect of cortical hormones on shock is in the case of severe burns, where plasma electrolyte changes seem to be prevented and the efficacy of a plasma transfusion is increased (146,147).

While considerable work has been done on the use of cortical extract and desoxycorticosterone in treatment of shock in the human, the reports are conflicting and difficult to assess (147,148,149,150,151,152). It is apparent that in very few instances has the degree of protection reported against shock been of sufficient magnitude to warrant the conclusion that the stress procedure had induced circulatory failure by reason of a sudden adrenal insufficiency.

ADRENAL CORTEX AND NITROGEN METABOLISM.

The process that is largely responsible for the direct relationship between nitrogen and carbohydrate metabolism has been named "gluconeogenesis", i.e., the conversion of protein to carbohydrate as seen in the maintenance of normal blood

glucose levels in the starving organism long after available carbohydrate stores have been depleted. Evidence for the direct participation of the adrenal cortex in nitrogen and carbohydrate metabolism lies mainly in the experiments which have demonstrated the necessity of its presence for normal gluconeogenesis to occur.

It has long been shown that the blood sugar levels of adrenalectomized animals was often low (153,154) and Britton and colleagues (155,156,157,158,159) in a survey of the symptoms which follow bilateral adrenalectomy, in a wide variety of species, showed an appreciable decline in body carbohydrate levels by blood sugar, liver, heart and muscle glycogen changes.

Long, Katzin and Fry (160) in their pioneering work, found, in agreement with other workers, that so long as adrenalectomized rats were maintained in good health by administration of sodium salt, no abnormalities could be observed in the storage of carbohydrate. When, on the other hand, the adrenalectomized animal refused food, or, when it was forced to fast, the liver glycogen showed a dramatic fall to extremely low levels accompanied by less severe but equally significant declines in muscle glycogen and blood sugar values. Not only could these changes be prevented by the administration of cortical extract, but the liver glycogen levels could be increased well above normal in these fasted animals, thus confirming previous observation of Britton and co-workers (157,158).

In careful metabolic experiments, it was observed that the increase in liver glycogen levels was accompanied by an increase in urinary nitrogen excretion, with the ratio of extra carbohydrate formed to extra nitrogen eliminated indicating that the glycogen increment could be entirely explained by a conversion of body protein to carbohydrate.

Evidence advanced by several investigators tends to support this general thesis that the action of cortical hormone may be more concerned with the catabolism of protein than the actual utilization of carbohydrate. Not only will adrenalectomy relieve the symptoms of severe diabetes in the pancreatectomized animal (161,162,163,164), but, as the glycosuria is reduced and finally eliminated, the level of urinary nitrogen also decreases (160,165,166,167). Cortical extract administration will increase the degree of glycosuria and also increase the urinary nitrogen. When phloridzin is given to the adrenalectomized rat, the excretion of glucose and nitrogen is but a fraction of that observed with the phloridzinized normal animal (168,169,170,171,172,173). The administration of cortical extract produces a marked loss in body weight, increases the glycosuria and also the amount of nitrogen excreted. Likewise, the rise in liver glycogen, accompanied by an increase in urinary nitrogen, which follows a short period of anoxia, is prevented by adrenalectomy (168,173,174) and can be restored with cortical extract injections.

While this relation of urinary nitrogen excretion to the changes in liver glycogen level appears clear, it should be

remembered that a decreased nitrogen output, accompanied by an elevation of blood nitrogen or protein nitrogen concentration is one of the earliest signs of adrenal insufficiency and denotes a decreased urea clearance by a hypofunctional kidney (175,196,177).

One should also bear in mind the observations of various investigators which point to the conclusion that abnormal amounts of carbohydrate are burned by the adrenalectomized animal. Evans (178) noted that when an adrenalectomized rat was fed glucose, it stored less of liver glycogen and used a greater portion than would an intact rat. Conversely, Russel (179) found that the administration of cortical extract to a glucose fed rat increased the proportion stored as glycogen and decreased the amount oxidized by the tissues. Katzin and Long (180) found a low R.Q. in such extract treated rats. Also, force fed depancreatized rats, given cortical extract show increased glucose excretion which cannot all be linked to protein catabolism. Conversely, the decrease in sugar excretion which follows adrenalectomy in the above, is not entirely related to the decreased conversion of protein to carbohydrate (166). All pointing to an inhibition of carbohydrate oxidation by either cortical extract or the intact cortex.

In man the correlation of adreno-cortical function with nitrogen metabolism has led to what has been termed the N and S Hypothesis (previously mentioned) and rests largely on metabolic studies on patients suffering from disorders of the

adrenal cortex as manifested by tumors or hyperplasia of this gland. Patients suffering from adreno-genital syndrome (usually associated with a cancer or adenoma of one adrenal cortex or with hyperplasia of both cortices), demonstrate an invariably high 17-Ketosteroid excretion, precocious and excessive and somatic development, indicating a state of increased nitrogen anabolism. On the other hand patients with Cushing's syndrome in whom the existence of cortical hyperplasia or carcinoma has been proven, an excessive nitrogen loss has been demonstrated by Albright and collaborators (181) accompanied by osteoporosis, atrophy of the skin and impaired glucose tolerance, with increased "cortin-like" substance in the urine (182) as measured by Selye-Schenker cold test (183). The last providing experimental evidence for relationship between cortex and nitrogen metabolism.

Using the above assay method as an index of adreno-cortical activity, Weil and Browne (184,185) demonstrated increased amount of cortin-like" substances in the urine of individuals who had been exposed to various kinds of damage. These findings were later confirmed by Dorfman (186). The maximal excretion of "cortin-like" material occurred at the time after damage which corresponded approximately with the period of abnormally high nitrogen excretion.

Adreno-cortical function has also been estimated in the manner described by Reinecke and Kendall (187) which measured directly the capacity of adreno-cortical steroids to influence gluconeogenesis, by the measurement of deposited

glycogen in fasted rats (or alternately mice) which have been previously adrenalectomized. Employing this method Venning, Hofrman and Browne (188) have reported increases of 3 to 13 times normal levels in patients having undergone various operative procedures.

From above considerations has emanated the "N" and "S" Hypothesis which may be briefly mentioned here again. An esposition of this view postulates at least two types of hormones are derived from the adrenal cortex, the "N" hormone - a nitrogen storing one and the "S" hormone which is anti-anabolic - i.e., inhibiting protein synthesis.

In the above discussion relationships between adrenal and other endocrine organs has been omitted nor has any attempt been made to review any of the other endocrines, which may directly or indirectly influence nitrogen metabolism - the relationships being too complex and as yet not fully understood. One must, however, in all fairness mention the all important role of the pituitary.

A relationship between it and nitrogen metabolism has been demonstrated in the works of Cuthbertson and his associates (189,190) in which it has been shown that crude extract of this gland have effectively prevented the increased nitrogen loss associated with the separation in time of the carbohydrate and protein moieties of the diet of adult rats and further the excessive excretion of nitrogen following fracture of femur in adult rats has been prevented. So that other mechanisms such as impaired pancreas function and pituitary dysfunction to mention a few, might directly and indirectly affect nitrogen metabolism.

EFFECT OF OTHER HORMONES

It was noticed by Cuthbertson et al (189) that a crude extract of the anterior pituitary tissue of the ox had definite nitrogen retaining properties when injected into the rat and further experiments showed that this particular extract, when injected daily into rats suffering from a fractured femur, produced by open operation, prevented the loss in body weight and excessive loss of nitrogen and creatine which are the usual concomitants of such injury. No greater rate of restitution of the atrophied muscles of the injured limbs was observed and further the extract had no significant effect on the total time required to heal superficial wounds (190). The effect of the extract on the metabolism of the injured animal was general rather than local, and possibly represented the normal growth response to this extract, superimposed on, and thereby masking the increased catabolism following injury.

It is interesting to note, at this point, that the feeding of dried thyroid accelerated the ratio of skin wound healing in rats (191).

Insulin:

In a series of exhaustive studies, Thomsen (191A) has shown that post-traumatic disturbances include upset carbohydrate metabolism, as manifested by spontaneous or alimentary glycosuria and hyperglycaemia, in a considerable number of injured persons. A review of the examinations of fasting blood sugar and glucose tolerance curves revealed

a tendency to deviation of the experimental results in an abnormal direction.

In view of the work of Selye (192) as to the response of the adrenal cortex to damage as evidenced by hypertrophy it might be interesting to speculate as regards an imbalance between insulin and the cortical hormone after trauma. It has been shown that the adrenalectomized animal is extremely sensitive to insulin, and that it can be protected by the administration of cortical extract (193) or adreno-cortical transplants (194), and Wells and Kendall (195) have suggested that cortical hormone specifically blocks the action of insulin.

It might seem therefore, that the period of negative nitrogen balance following damage might be due in part, at least, to an imbalance between the pancreas and the cortex. No studies have as yet appeared in the literature as to the amelioration of this excessive nitrogen loss by insulin administration.

MALE SEX HORMONES AND PROTEIN METABOLISM

That numerous endocrine glands affect directly or indirectly protein metabolism is evident from the previous discussion regarding the thyroid, anterior pituitary extract and the role the adrenal cortex plays.

It has been demonstrated as early as 1935 (196) that male sex hormones extracted from urine consistently led to nitrogen retention when administered to castrated dogs, the decrease in urinary nitrogen being entirely due to the urea fraction. It was found that the nitrogen retained was never completely excreted following discontinuation of the hormone treatment, in contrast to nitrogen and sulphur stored by giving excess food to normal persons and therefore, here, part of the nitrogen is taken up structurally and not temporarily stored. This effect was exerted in castrated dogs by natural and synthetic androgens and by activated natural products, the energy metabolism remaining unaffected.

Kenyon, Knowlton and Sandiford (197) demonstrated similar nitrogen retention when testosterone propionate was administered to human males with eunuchoidism, the change being again a decrease in urea excretion without alteration of the nitrogenous constituents of the blood and it was noted that there existed a maximum intensity of nitrogen retention per day in response to the administered androgen, which could not be augmented by increasing the dosage or prolongation of treatment. Subsequent studies by Kenyon et al, (198) showed the same effect of testosterone propionate in normal males,

although it was quantitatively less marked than in eunuchoids. It has been shown by Browne and Ross (199), Webster and Hoskins (200) that the administration of testosterone propionate to young boys with retarded growth resulted in a remarkable increase in growth rate and body weight.

Kenyon and co-workers (198) in an excellent detailed review, evaluate the experimental and clinical evidence, the latter afforded by observations on patients with androgenic tumors, which establish the anabolic effect of androgen, particularly on protein metabolism and their somatotropic effect on man.

They suggest that the skeleton and skeletal musculature, possibly the kidneys and other organs, may be the nongenital site of new tissue deposit under the influence of androgens. However, the response noted in normal males are interpreted as indicating that the mature normal testis is not exerting this anabolic or somatotropic effect to the fullest extent to which the organism can respond. Since the aging process in itself does not appear to hurt the capacity of the organism to respond to the metabolic effect of androgens, it is reasoned that some as yet obscure opposing process must counterbalance the anabolic effect of the normal testis in order to achieve the nitrogen equilibrium characteristic of maturity. Otherwise, a positive balance of the affected tissue constituent would be apparent throughout adult life. That this mechanism might be in the adrenal cortex is not suggested in the above paper, but it may well be.

Albright and Browne (201) have postulated that the adrenal cortex produces an "N" or nitrogen or protein anabolic formone and an "S" or sugar or anti-anabolic hormone. These hormones are normally balanced, but after trauma has occurred there is an initial phase lasting 24 to 48 hours in which there is an increased excretion of both "N" and "S" hormones, followed by a second phase lasting days, weeks or even months, in which there is decreased excretion of "N" hormone and an increased excretion of "S" hormone. Eventually, if the organism survives, the two hormones come into balance, and finally there may be a compensatory phase where the "N" hormone production is increased at the expense of the "S" hormone. Testosterone would appear to fulfill the role of the "N" hormone.

No known attempt has been made in damaged subjects to investigate the possibility of lowering the abnormally high nitrogen excretion during the period of heightened nitrogen excretion, by the administration of androgenic compounds, although in the opinion of D. P. Cuthbertson (202) "In the light of previous experience the writer doubts that testosterone therapy will achieve more success that that achieved by simply inducing nitrogen retention with anterior pituitary gland extract".

GENERAL SUMMARY OF HISTORICAL REVIEW

It has long been recognized that patients with a variety of infectious diseases lose protein at a rapid rate as evidenced by very high urinary nitrogen levels - (203,204, 205,206,207,208,209,210,211). Most early observers agreed that the negative nitrogen balance regularly observed in these conditions could be alleviated, but not prevented, by high calorie diet (up to 5,000 calories of carbohydrate and fat). This gave rise to the general opinion that it was obligatory and unpreventable, and is reflected in the term, "toxic destruction of protein".

In a series of publications dating back to 1930, Cuthbertson has reported studies of metabolism of convalescing patients in whom fractures had occurred (60,117,118,119,139). He and his associates observed large losses of body protein. The source of this lost protein and the mechanism of the catabolism have been matters of considerable interest, although until recently they have received little study by other investigators. Cuthbertson (202),and Browne, Schenker and Stevenson (212,212a,213) and others (214,215,216) have found that the nitrogen excretion in the succeeding three days after operation or fracture rises to a level distinctly above that usually encountered in starvation, reaching a peak usually during 4th to 8th day after the injury to the organism. The duration of this protein destruction after simple operations is, however, short; positive nitrogen balance with replacement of lost protein sets in early and the total loss of protein, if the diet is adequate, is small.

This work has been confirmed and extended by others in this laboratory, Schenker, Stevenson and Browne (212,213) have demonstrated that in the human adult in good health and nutrition, a damaging stimulus is followed rapidly by a disturbancein the nitrogen metabolism in the direction of catabolism; this catabolic response was shown to occur in such patients irrespective of the type of injury.

The work of Peters (220) in infections, especially meningitis, and Cuthbertson, Browne et al (202,213), and Howard (215,216) in all sorts of operative procedures and fractures have shown that unless relatively enormous quantities of protein nitrogen, together with high calorie intakes are ingested during early stages of this catabolic response, a negative nitrogen balance persists. In fact, Peters (126) concludes that "The negative nitrogen balance was not perceptibly influenced by the protein intake".

From the work of Cuthbertson (117,118,119), Browne (212,212a,213), Howard (214,215,216), and others, it appears quite clear that in healthy, vigorous male patients with fractures, there is set up in response to trauma a reaction which results in vigorous "protein" catabolism with losses to the organism of the nitrogenous portion (as urea) of large amounts of body protein. This dynamic process seems to diminish progressively during normal convalescence. At the height of this reaction (protein catabolic process or period) the effect of increasing the intake of highest quality protein and calories, namely conversion and storage (60) is not

detectable, as increments in the nitrogen intake are paralleled by similar increments in nitrogen output, mainly as urea. And during this period of protein catabolism, a fracture patient on a low protein, low calorie diet does not seem to lose any more body protein than do similar patients on higher nitrogen and caloric diets.

This reaction "protein catabolic process" is obviously unlike the reaction in starvation, where the expenditure of "endogenous protein stores" may be readily suspended by provision of either adequate protein or calories. Nor can the destruction of protein be attributed merely to accelerated energy expenditure or heat production. Since high caloric diets prevent excessive nitrogen loss both in exercise (209) and in hyperthyroidism (210,211,217,218).

It is conceivable that the course of protein metabolism is distorted after injury, and it has been suggested that certain amino acids or acid are diverted from their normal paths during the processes of repair.

A recent report by Peters and Croft (141) claims "That the urinary losses in nitrogen after burning were reduced substantially by the inclusion of 1% dl-methionine in the diet and that neither alanine nor a mixture of synthetic amino acids, not cystine, had a similar effect". The above results were not confined with burned patients in this laboratory (219).

It would seem that the type of reaction occurring in fracture patients demands of the body that a certain amount of "endogenous" protein be catabolized and if additional protein

beyond the minimum is fed it is deaminized and without exerting any sparing effort whatever on the body nitrogen.

The significance of this accelerated protein catabolism after injury is not yet clear. That absorption of protein, during this period, is unimpaired is attested by innumerable analyses of stools (203,204,205,209,220 and 131). In any case, impaired absorption could not explain excessive urinary nitrogen nor failure to retain parenterally administered protein hydrolysates as shown by Browne et al (221).

It has previously been stated that the degree to which the catabolic response occurs following damaging stimuli is governed by the state of health and the nutrition of the organism prior to damage. It has been shown in this laboratory (116) that patients who have been chronically ill or in a state of malnutrition (debilitated) for some time prior to being exposed to a damaging stimulus, show little or no catabolic response and are able to retain nitrogen at relatively low intakes of protein nitrogen and calories. Howard reported (216) a case who was cachectic from long standing rheumatoid arthritis at the time of multiple fractures, who failed to show any increase whatsoever in his protein catabolism. In this debilitated state when they suffered renewed trauma or developed infections, some of Browne's (221) patients continued to store nitrogen.

The impression is acquired that a healthy man subjected to acute infection or injury suffers protein depletion that cannot be prevented by any dietary measures thus far discovered; on the other hand in the subject who is malnourished and who

has already been depleted of protein there are certain mechanisms that permit him to utilize protein for possible tissue reconstruction. It may be, therefore, that this "wastage" (i.e., in previously healthy individuals) may be associated with the process of repair rather than the destruction arising from the actual injury and the catabolic process may be a desirable and useful one which debilitated patients are unable to accomplish.

The work reported in this thesis arose out of an interesting report by Cuthbertson (78) that the nitrogen excretion did not rise in injured rats who had previously been fed a protein-free diet until a steady level of nitrogen excretion had been reached; whereas, in normally fed rats similar injury elicited a well marked loss of urinary nitrogen. Whereupon, it was concluded that the nitrogen losses after fracture arise from labile (deposit) protein stored away before injury. It was felt that the old concept of "deposit protein" has been too much discredited in recent years and that reviving it now was hardly appropriate since in the catabolic phase of injury exogenous protein (amigen,etc.) (221) seems to suffer the same fate as endogenous protein.

It was thought that the lack of response to injury on the part of the "Protein Depleted Rat" may have directly or indirectly involved a lack of response to damage on the part of the adrenal cortex.

It has been postulated by Albright and Browne (201) that the nitrogen balance following trauma is associated with

increased production of substances which cause gluconeogenesis, derived presumably from the adrenal cortex. The increase of these substances in the urine has been demonstrated by Weil and Browne (184,185) and by Venning, Hoffman and Browne (188).

There is some suggestion in a recent report by Dobriner (222) that such an increase does not occur in the urine of patients with cancer when operated upon. It has also been shown (223) in one or two cases that the 17-Ketosteroids of seriously depleted patients are very low.

These findings suggest the possibility that the difference in response between a healthy and chronically ill, depleted person or animal (organism) might be due to a failure on the part of the adrenal to put out gluconeogenic substances after damage, rather than to a depletion of "labile" protein stores. It was with this view in mind that the following experiments were undertaken.

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

Male hooded rats weighing between 340-350 grams were used in all of the experiments, the weight range being kept as constant as possible.

Standardization of methods so that there might be the least possible change in experimental conditions, was deemed of utmost importance, as the significance of these metabolic studies lies in the relative changes in urinary metabolites examined from day to day. Therefore, before any studies were commenced on an animal it was allowed a period of 3-4 days to adapt itself to the new surroundings of the metabolism cage (224). The modification of the cage will be described later.

The collection of urine was done every 24 hours and, to be assured not only of completeness of urine specimens, but also that the daily specimens should be representative of not more or not less than of one 24 hour period, each collection was completed by slight pressure on the rat's abdomen to effect emptying of the bladder. Also creatinines were determined daily to check completeness of specimen.

The rats were in individual metabolism cages (224) with the glass grid modifications as described by Heller (225), which was effective in separating feces from urine. The urine was collected in a graduate on which rested a glass funnel, the graduate containing a few drops of glacial acetic acid to prevent any escape of NH_3.

Each collection period (24 hours) the rats were moved to clean metabolism cages and were weighed on a trip

balance during this process. The cage was then washed down with 2% tartaric acid. The volume of acid used was about 50 cc., and final volume adjusted to 100 cc. This was then filtered into 100 cc. graduates. The nitrogen content of the urine was then determined on 2 cc. aliquots of the diluted urine and the creatinine on 0.5 cc. aliquots. The tartaric acid used assured no solution of feces (120). This was checked where feces specimens of 24 hours duration from two rats were allowed to stand for 8 hours in 50 cc. of tartaric and only approximately 2 mg. of nitrogen were leached out, an insignificant amount.

TABLE I

	Nitrogen leached out from feces by tartaric acid	
1.	50 cc. 2% tartaric	0 mg. nitrogen
2.	50 cc. 2% tartaric standing over 24 hours feces collection of two rats for 8 hours	2.1 and 2.4 mg. nitrogen

No account was taken of nitrogen lost by hair as this has been shown by Voit (226), to be of no significance. The temperature of the room in which the metabolism cages were kept showed initially in the first experiment some fluctuations, in the range of 5° F., but this room was provided with a thermostat and the temperature was maintained around 75° F.

The diet box of the metabolism cage (224), made of galvanized iron, is divided into two compartments. One forms a bin of wire mesh, for gross food; the other, with a dividing shelf with hole in center, allows tip of water bottle to pass through. Below these two compartments is sufficient space to insert a two-compartment drawer which is used to hold ground food on one side and to catch drips from the water bottle on the other.

The nature of feeding and determining the food intake, was to weigh out given amount of food (in form of pellets) with the drawer, and then quantitatively transfer it into the food bin with the wire mesh. At end of each metabolic period the food left over is then replaced in the drawer and weighed, the difference representing the food intake. The amount of food lost in powder form over the cage, by the animal taking small amounts into its paws, proved negligible.

The above method of feeding was decided upon rather than that used by Cuthbertson et al (120). Cuthbertson placed the rats in a feeding box for 2 hours out of every 24 hour period and corrected for urine lost by multiplying days total with 24/22 as correction factor. This obviously lends itself to greater error, as in one case the rat may not urinate, and in another almost one third or more of day's specimen may be lost. Even though a constant food intake would have been preferred, forced tube feeding was not resorted to, as this would introduce the complicating factor of all the food being ingested at once.

In view of the fact that a little powdered food was lost onto the monel metal funnel and into collecting graduate it was decided to determine whether any nitrogen from the normal protein diets would be leached out in the washing with 2% tartaric acid or in the urine alone. As is demonstrated in the table below, an almost imperceptible amount of nitrogen was leached out from either Purina or the low "K" 20% protein diet. 100 cc. of diluted rat urine was divided into two 50 cc. batches and one batch shaken up with powdered Purina. Another 50 cc. 2% tartaric acid was shaken up with powdered low "K" diet. All samples were allowed to stand for 24 hours.

TABLE II

Nitrogen leached out from Purina and low "K" diet as mgs. Nitrogen/50 cc. fluid

Plain urine sample	5.48	5.41
Urine with Purina	5.91	5.96
" " low K diet	5.63	5.61
2% tartaric acid with Purina	0.91	0.93
" " " with low K diet	0.83	0.87
Tartaric (blank)	0.05	0.052

That no significant loss of nitrogen occurs during the process of collection of the urine is shown by the following experiment. 20 cc. of rat urine, whose nitrogen content had previously been determined, were dropped from graduates into each of four cages over a period of five hours. After this period the volumes of urine that had been collected in the graduates were measured and then the cages were washed down with ca. 75 cc. tartaric, as was done in the metabolism experiments. The recovery of fluid was only from 75-85% of the amount dropped into the cages.

The amount of nitrogen recovered after washing down the cages was from 98-99% of the original amount of nitrogen. These results are shown in Table 3.

TABLE III

Recovery of fluid and nitrogen after dropping 20 cc.urine into cages

Cage No.	Original N content mgm.	cc. urine recovered	% volume recovered	mgm. N recovered	% N recovered
1	400	15.	75.	392.3	98.08
2	400	16.3	81.5	394.5	98.6
3	400	14.8	74.0	392.0	98.0
4	400	17.2	86.0	396.4	99.1

So that in the recording of urine volumes in all probability some of the fluid weight has been lost in evaporation. Although the nitrogenous substances thus crystalizing out would be recovered with the tartaric acid washings.

When speaking of "damage" in these experiments one refers to open fracture of the left femur. The method employed was open fracture under nembutal anaesthesia, involving a careful, blunt dissection of overlying muscle groups (to incur as little muscle damage as possible) and exposure of femur, cutting with heavy scissors and removing any small bone particles. This was then sutured without application of splints. All adrenalectomies were performed by the usual dorsal approach, using a single incision and ether as anaesthetic.

Chemical Methods:

I. Determination of total nitrogen

The method used in these studies was a semi-micro modification od the standard Kjeldahl procedure carried out on aliquots containing up to 15 mg. of Nitrogen. Digestion was

effected by the use of selenium dioxide and copper sulfate as catalysts, concentrated sulfuric acid as the oxidizing agent, and phosphoric acid as a means of raising the boiling point of the digestion mixture. The latter was prepared by mixing the constituents together and adding 2 cc. of the sample to be analyzed. Digestion was carried out on a rack fitted with 6 micro-burners, and with the mixture described, complete digestion (until solution colorless) was achieved in about 10-15 minutes.

Distillation was carried out using 6 Liebig condensors mounted on stands and fitted with Kjeldahl bulbs which could be attached directly to the 300 Ml. Kjeldahl flask by means of a rubber stopper, this enabling six distillations to be carried out simultaneously. Instead of distilling the ammonium hydroxide over into standard acid, Heidelberger's modification of the method of Meeker and Wagner (227) was used. The modification consists of a change in the original indicator (methyl red) to one composed of 125 parts of a saturated solution of methyl red in 95% ethanol and 15 parts of a 1% aqueous solution of methylene blue. ca. 15 mls. of a 3% (-) solution of boric acid was employed as receiver. Calculations are greatly simplified by the use of standard N/14 sulfuric for titrating. The volume in ml. required for titration then represents the number of mgm. of nitrogen contained in the aliquot of the specimen analyzed. Control analysis of glycine gave recoveries within 1% of the theoretical.

Determination of preformed creatinine

The Carphin-Birmingham (116) modification of the standard Folin method was used. The modification consists essentially of increased sensitivity by the use of smaller volumes of urine and picric acid mixed directly in colorimeter tubes graduated at 40 ml. The volume of urine used was 0.5 ml. of the 100 ml. total and gave readings within the range of 50-75 on the Evelyn Colorimeter tube calibrated to contain 40 ml. and using a standard 1 ml. pippette graduated to 0.05 ml. 8 cc. of 94% picric acid solution are then added, followed by 0.6 ml. of 15% sodium hydroxide. A micro-burette is used for the latter, and a 50 ml. capacity burette graduated in 0.1 ml. for the picric acid (recrystallization of the picric acid was found unnecessary; commercial picric acid of highest purity is adequate). The contents of the tube are well mixed and allowed to stand for exactly 10 minutes after which the total volume is made up to 40 ml. with distilled water. In order to allow color development to reach the equilibrium, another 15 minutes are allowed to elapse before reading on the Colorimeter. During this latter interval, the tubes are inverted 10 times to ensure complete mixture. A center setting is obtained by means of a blank tube containing reagents only and treated in the same manner as the tubes containing the urine samples.

The creatinine contents of the sample is obtained from a table derived from a calibration curve (116). A filter with normal transmission at about 520 multi-microns is used in the Colorimeter.

All determinations of nitrogen and creatinines including the blank in the latter, are done in duplicate.

Determination of NPN

Sulfate Tungstate Sol. 75 grams Sodium Sulfate) Made up to
30 " " Tungstate) 5 liters

1 cc. blood gently allowed to drop into 8 cc. of the sulfate. Tungstate Solution then inverted and allowed to stand for 10 minutes. Then 1 cc. of 1/3 N sulfuric acid is added to the above, inverted again three times and immediately centrifuged for 5 minutes. A Micro-Kjeldahl is done on 5 cc. of the supernatant solution. This involves exactly the same procedure as in the semi-micro procedure, except the standard Micro-Kjeldahl apparatus is used for distillation and 1/70 N sulfuric is used for the final titration.

D I E T S

Low Protein Diet

This diet was devised to contain as low a protein content as possible with adequate amount of all other factors, viz., vitamins, minerals, fat and carbohydrate.

	Gm.	% Protein	% Fat	% CHO
Potato Starch	50	0.6	-	83
Rice Starch	10	9	6	68
Cane Sugar	10	-	-	10
Butter	5	-	86	-
Salt Mixture	5	-	-	-

Which gives 3.8 cal./gm.

1.5% protein by weight Nitrogen Content 1.21 mg.N/gm.

Vitamins

Alphatocopherol	4 mg.	per 80 mg. ration
B_1 (Thiamin)	1 "	" " " "
B_2 (Riboflavin)	3 "	" " " "
B_6 (Pyridoxine)	2 "	" " " "
Ca Pantothenate	2 "	" " " "
Choline	2,000 "	" " " "
Nicotinic Acid	200 "	" " " "

Cod Liver Oil Supplement

Salt mixture consisted of following and was styled after that of Steenbock and Nelson (228).

COMPONENT	GM.
$CaCO_3$	26
$3MgCO_3.Mg(OH)_3.3H_2O$	6.1
HCl	22.4
$NaH_2.PO_4.H_2O$	16.8

Salt Analyses (Cont'd.)

Na_2CO_3	20.6
$Fe_2(NH_4)_2$, $(C_4H_5O_7)_2$,$3H_2O$	4.0

PURINA (Fox Chow)

Purina diet analysis as shown below is that of the manufactuere's analyses except for nitrogen content which checked to within 2% of that provided by the manufacturer. As the possibility exists that different batches of Purina received in this laboratory might vary slightly in at least its nitrogen content, analyses were conducted on different samples from time to time and were found to vary only within 1% (±) range.

	Crude %	Digestible
Protein	23.0	19.0
Fat	5.0	4.7
Fibre	4.0	
Ash	7.0	
Nitrogen Free Extract	54.0	48.0
Moisture	7.0	

Caloric Value - 3.5 cal/gm. Nitrogen Content 45.0 Mg/Gm (Writer's analysis)

Mineral Analysis of Purina

	% by weight
Iron	0.018
Magnesium	0.09
Silica	0.23
Potassium	0.56
Sodium	0.67
Chlorine	0.78
Phosphorus	1.17
Calcium	2.22

(non-goitrogenic)

Vitamine Analysis of Purina (Fox Chow)

Vitamine A — ca. 4000 International units per pound

B — 275 Sherman units per pound, supplied by wheat germ oil

C — Low

D — ca. 500 International units per pound, supplied by cod liver oil

E — No measurement - but supplied by richest known source, viz., wheat germ

B_2 — 300 Sherman units per pound

LOW K DIET

And finally the following diet was constructed to afford adrenalectomized rats a low potassium and high sodium intake as it was thought that the high K content in Purina might prove deleterious to animals sunjected to this operation. The salt mixture is the same as that used in the low protein diet except for equimolecular replacement of KCL by Nacl.

SPECIAL DIET FOR ADRENALECTOMIZED RATS

	Gms,	Protein %	Fat %	CHO %
Casec	15	88.0	-	-
Rice	38	8.6	6.1	68.0
Corn Starch	35	-	-	90.0
Brewer's Yeast	8	48.0	2.0	39.0
Salt (Low"K")	5	-	-	-
Cod Liver Oil supplement	-	-	-	-

Diet contains: 21% Protein

" " 3.47 cal. per gm.

Nitrogen Content: 40.1 mg/gm.(Writer's analysis)

SUZA'S SOLUTION

	Gms.	ccs.
Mercury Bichloride	225	-
Glacial Acetic Acid	-	200
40% Formaldehyde	-	1,000
Distilled Water	-	4,000
Sodium Chloride	25	-

EXPERIMENT I

(Rats 1-8 incl.)

In this experiment an attempt was made to repeat the experiments reported by Cuthbertson and Munro (78). Two sets of 4 rats were employed. One set was maintained on a diet of Purina fox chow, which can be considered to contain about 20% Protein and the other set of four on an almost Protein-free diet, i.e., 1.5% Protein content (by weight).

Each group of four rats was divided into two groups of two each according to weight range, one set of two weighing in the vicinity of 300-325 grams, the other group in the range 260 - 10 grams.

Metabolic studies on 24 hour specimens involving the measurement of total nitrogen and creatinine levels were carried out, the creatinine serving as a check of the completeness of the urine collection. Daily measurements of urine volumes and weights of the animals were also done. The plotted results are shown in Figs. 2-5 inclusive.

As previously discussed the method of "ad libitum" feeding was decided upon in view of the failings of other methods, even though this involves serious limitations, such as failure to ensure a constant caloric and nitrogen intake.

An examination of the daily urine volume levels fails to reveal any correlation between this and the urinary nitrogen levels and one must also bear in mind that the urine volumes measured as such in all probability do not reflect the true volume of urine excreted, as some (10-20%) evaporates on the monel metal funnel as was shown in control collections.

Urinary nitrogen levels were followed until the 23rd day of collection, when on this day the left femur of all the rats (1-8) was fractured by the method described under the section on methods.

No values were recorded for the day of operation but the results were followed for a period of nine days after.

Exactly the same procedure was employed in the metabolic studies of the rats fed the "low" protein diet. The 23rd day was chosen for the operation because it was prior to this point that the both groups (purina and low protein fed rats) had already reached and maintained a steady level of nitrogen excretion (within certain small limits).

EXPERIMENTAL RESULTS AND DISCUSSION

It will be seen that the daily fluctuations in the level of urinary nitrogen are quite large in all rats in the first 10 days and may be attributed to the lack of standardization of collection technique and familiarity with same on the part of the observer and also possibly to the capriciousness of the animal's appetite up to this point, i.e., until the animal is adapted to the new surroundings and a steady intake is maintained.

There occurs a marked rise in excretion of nitrogen in the animals (rats 1-4) fed purina, the mean loss averaging 0.510 gm. nitrogen in the nine days post-operative.

The curve of nitrogen excretion in these animals (rats 1-4) is of interest, and repeats the characteristic form already noted by Cuthbertson (120) in the cases of both humans and in adult male rats. There is a gradual rise to a maximum on the 3rd-4th day and then a gradual fall, to be succeeded

in some cases (Figs. 2 and 3) by a small secondary rise some 8 days after injury.

The nature and reason for this small secondary rise is unknown and the output of creatinine remained remarkably constant in these experiments.

Cuthbertson et al (120) have shown that these effects are not due to the anaesthetic and they also found that the actual fracture of bone is not the determining cause of this metabolic disturbance, although undoubtedly a factor in prolonging it.

As opposed to the rise in urinary nitrogen noted in the purina fed rats no rise comparable to the above was noted in the rats fed a low protein diet. Although a very slight rise in nitrogen output may be noted in rats 5 and 7 in the first two days after damage, it will be seen that after the second day post-damage in all rats fed a low protein diet there occurs a steady slight decline in the level of urinary nitrogen output which may well be a continuation of that seen before trauma. In all rats caloric intake was well maintained after damage, i.e., the intake was maintained at the same level as that prior to damage, although the needs in both protein and calories, may be considerably above this after trauma.

Cuthbertson and Munro (78) found that it took about 14 days to reach a steady low level of urinary on a protein-free diet. Ashworth and Brody (229) reported that the urinary nitrogen per kg. body weight excreted by adult male rats living on a nitrogen free diet, did not reach a minimum value until 10-15 days after the beginning of the diet, and that thereafter

the daily variations were large, whereas Roche (230) finding similar results with some rats, found in other rats that a sudden rise in urinary nitrogen occurred, immediately preceding the death of the animal from nitrogen starvation.

Results similar to Roche's have been found in two cases and will be reported later.

In the metabolic studies on the low protein requirements it will be seen that in rats 5, 6 and 8 it required approximately 20 days for a steady and maintained low level of nitrogen excretion to be reached. That is required this period of time may be associated either with the 1.5% protein content of the diet (albeit of poor biological value) or with the individual idiosyncrasies of this strain of rats, or both.

It may be also noted that in one rat (No.6) an infectious sore just above the penis was noted on the 22nd day. There was no accompanying rise in urinary nitrogen in the succeeding 8 days during which the infection peristed.

In the rats fed a Purina diet (rats 1-4) all but No.3 maintained their original weight for a period of ten days prior to the damage. Although a weight loss in some of these may be seen at the beginning of the experiment, it was later regained and a steady weight maintained thereafter until the trauma was inflicted. After the trauma the weight loss in the Purina fed rats averaged 15-20 grams per rat, an amount similar to that observed by Cuthbertson (120).

It is well known that an adequate protein intake, both in quantity and quality, is necessary for maintenance as well as growth. The lack of the aforementioned is reflected in the steady

weight loss of all the rats (5-8) fed the low protein diet. An interesting feature is that the rate of this weight loss is not accelerated after damage. An observation in keeping with the lack of increase in urinary nitrogen excretion during this period.

Munro and Cuthbertson (78) concluded in view of the fact that the rats fed protein-free diet for 14 days failed to show an increased output of urinary nitrogen after damage that "The excessive output of nitrogen following injury arises from storage protein and not from essential tissue substance".

It will be seen from Figs. 3 and 4 that including the values of fecal nitrogen (10% of nitrogen intake), these rats are still in negative notrogen balance and are therefore still losing, presumably, body protein. This being so, why should the body discriminate in its handling of its so-called "storage protein" and the nitrogen precursors after damage in the organism fed the low protein diet, a view hardly in keeping with the work of both Schoenheimer (18) and Tarver and Schmidt (57).

T A B L E

AVERAGE NITROGEN BALANCE AFTER DAMAGE IN "LOW PROTEIN DIET" RATS

	(Average) N INTAKE	(Average) URINARY N	FECAL N	MEAN N BALANCE
Rat 5	15.9	43	1.59	-28.7 mg.
7	15.4	43.4	1.54	-29.5
8	16.6	40.6	1.66	-25.7

Values averaged for 8 days after damage.

On the other hand the possibility may exist that the animals fed the low protein diet have adjusted their metabolic

economy (35) such that the metabolic precursors which lead to the high urinary nitrogen levels in the organism fed the Purina (or normal protein diet) are now reconverted and that the negative nitrogen balance seen after damage in these animals reflects the minimum body protein drawn upon to meet its daily metabolic requirements.

That the level of caloric intake is important is shown in the work of Cuthbertson (120) where he fed supplements of carbohydrate to rats already receiving an adequate protein diet, viz., Purina, and continuing on this dietary regime the rats were able to show a slight net gain of nitrogen in the 9 days after trauma. Also Roche points to the "premortal rise of nitrogen" in starving rats which had previously reached the "endogenous" levels.

Another possibility is the suggestion that the failure of "Low Protein Diet" rats to show post-traumatic increases in urinary nitrogen lies in the failure of the adrenal cortex to respond to the traumatic procedure.

The usual response of the cortex in damage and the N and S hypothesis may be recalled at this point.

The lack of response on the part of the cortex might be due to impaired function of cortex per se, or a hypofunctioning hypophysis.

Chronic inanition has been found to result in a diminution of the weight of the adrenal glands in a manner similar to that following hypophysectomy. The shrinking in size of the adrenal glands is due to atrophy of the cortex, the medulla remaining virtually unaffected (231). It has been maintained

by Mulinos, and Pomerantz (232) that the similarity of the effects of hypophysectomy and chronic underfeeding upon certain of the endocrines is due to the fact that inanition causes depression of the anterior pituitary and so the smaller size of the adrenal cortex in hypophysectomized animals (233) or during chronic inanition (232,234) is probably associated with the absent or diminished hormonal function of the hypophysis.

In a review of the above, Mulinos, Pomerantz and Lojkin (235) examined the effect of complete starvation, chronic inanition and of old age on the adrenal. They found that regardless of the age of the rat, chronic inanition as well as hypophysectomy, resulted in a lowering of the weight of the adrenals, the weight loss being restored in both cases by pituitary implants and further during complete starvation there was an increase in the weight and in the ascorbic acid content of the adrenal glands in both female and male rats.

The feeding of a low protein diet may be likened to chronic inanition, although here the limiting factor in the diet is its protein content. In later experiments it will be shown that the feeding of this low protein diet (1.5%) also results in marked adrenal atrophy which upon histologic examination was shown to be limited to the cortex.

Assuming that the degree of atrophy represents the state to which the cortex decreases its functional level then it may well be that in the rats fed the low protein diet the resultant cortical atrophy has impaired cortical function to the extent that in response to trauma the increased output of gluconeogenic substances (184,185) fails to occur.

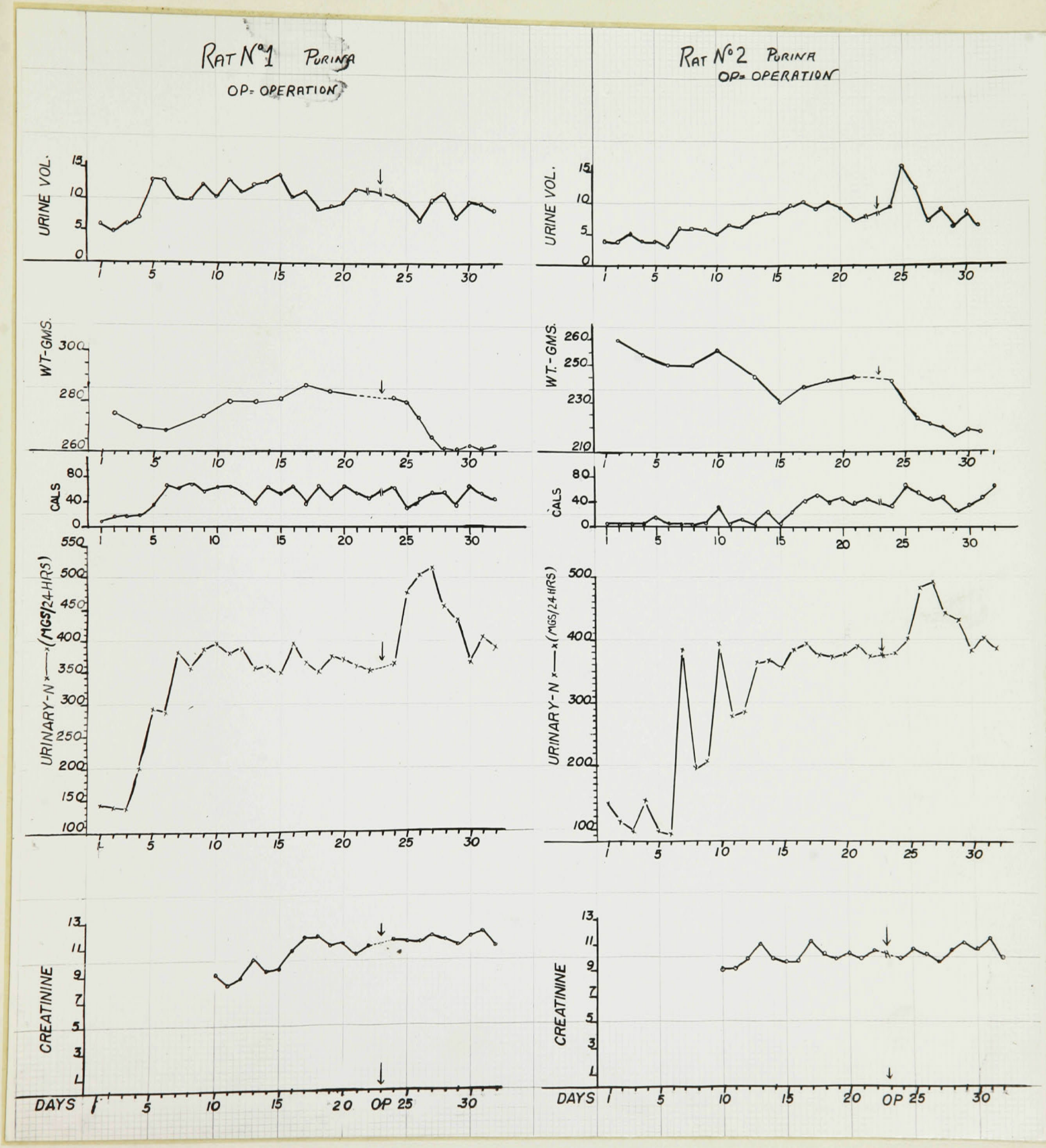

FIG. 2

Rats fed Purina and damaged on 23rd day

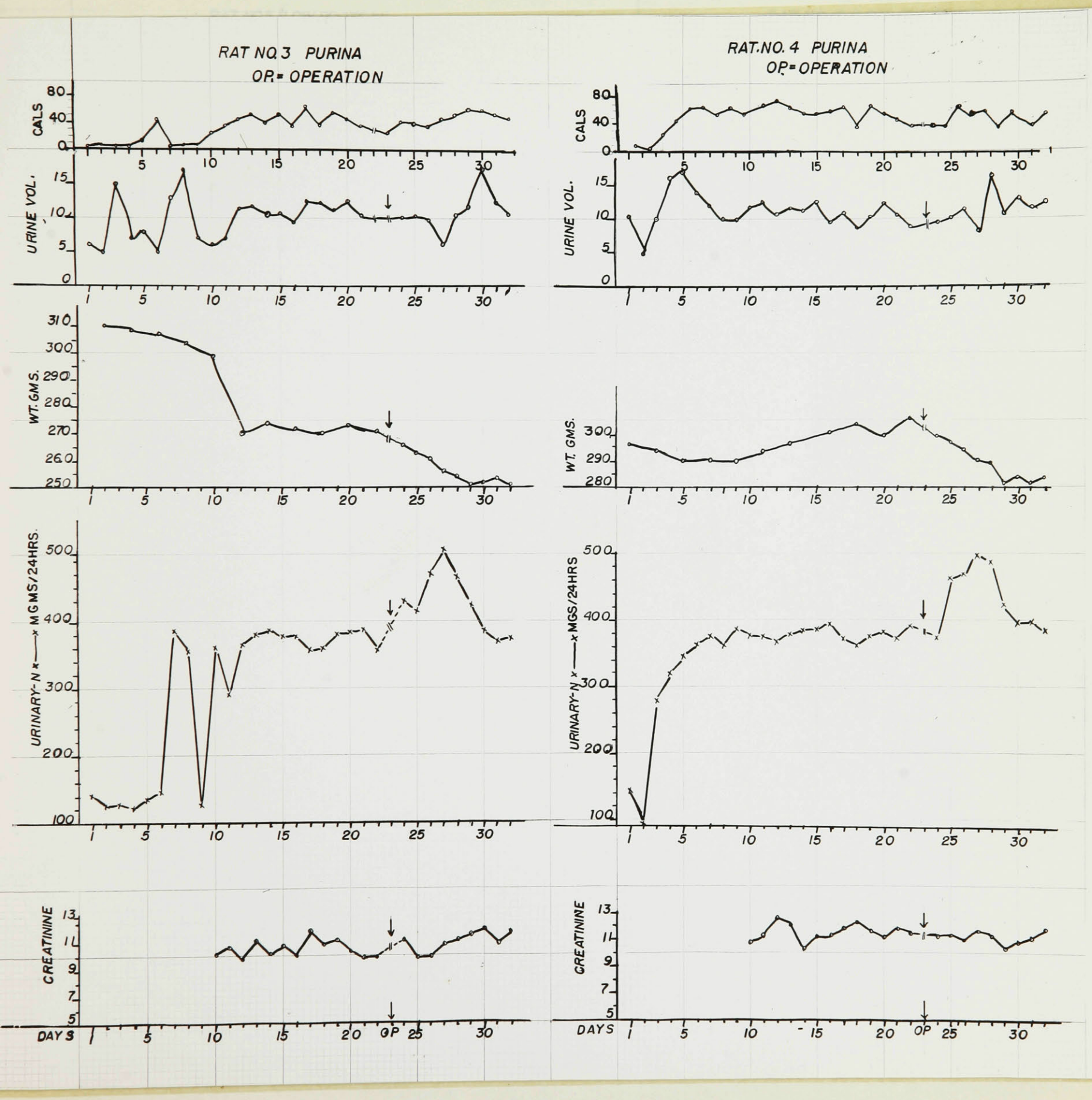

FIG. 3

Rats fed Purina and damaged on 23rd day

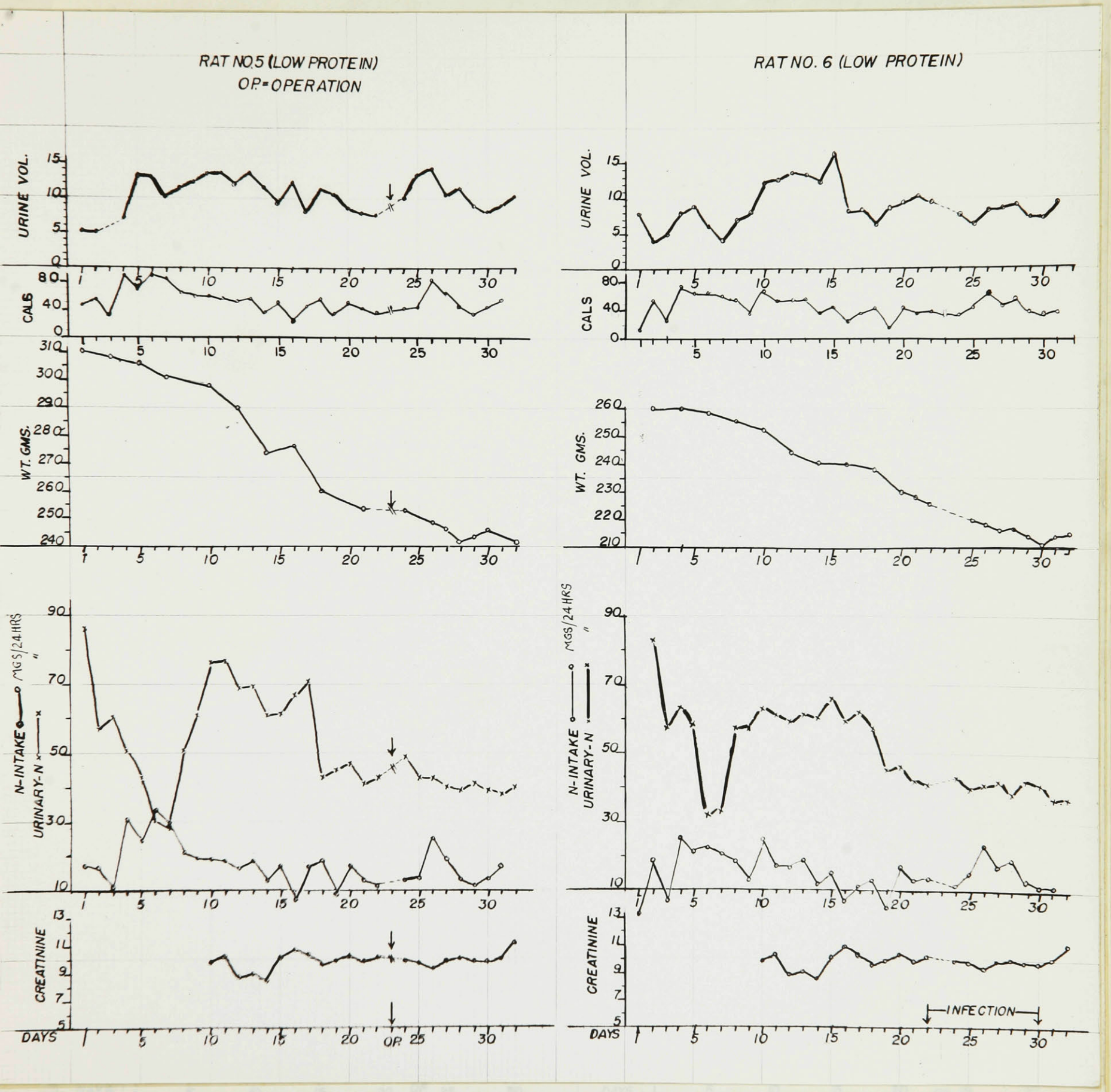

FIG. 4

Rats fed a low protein diet and damaged on 23rd day

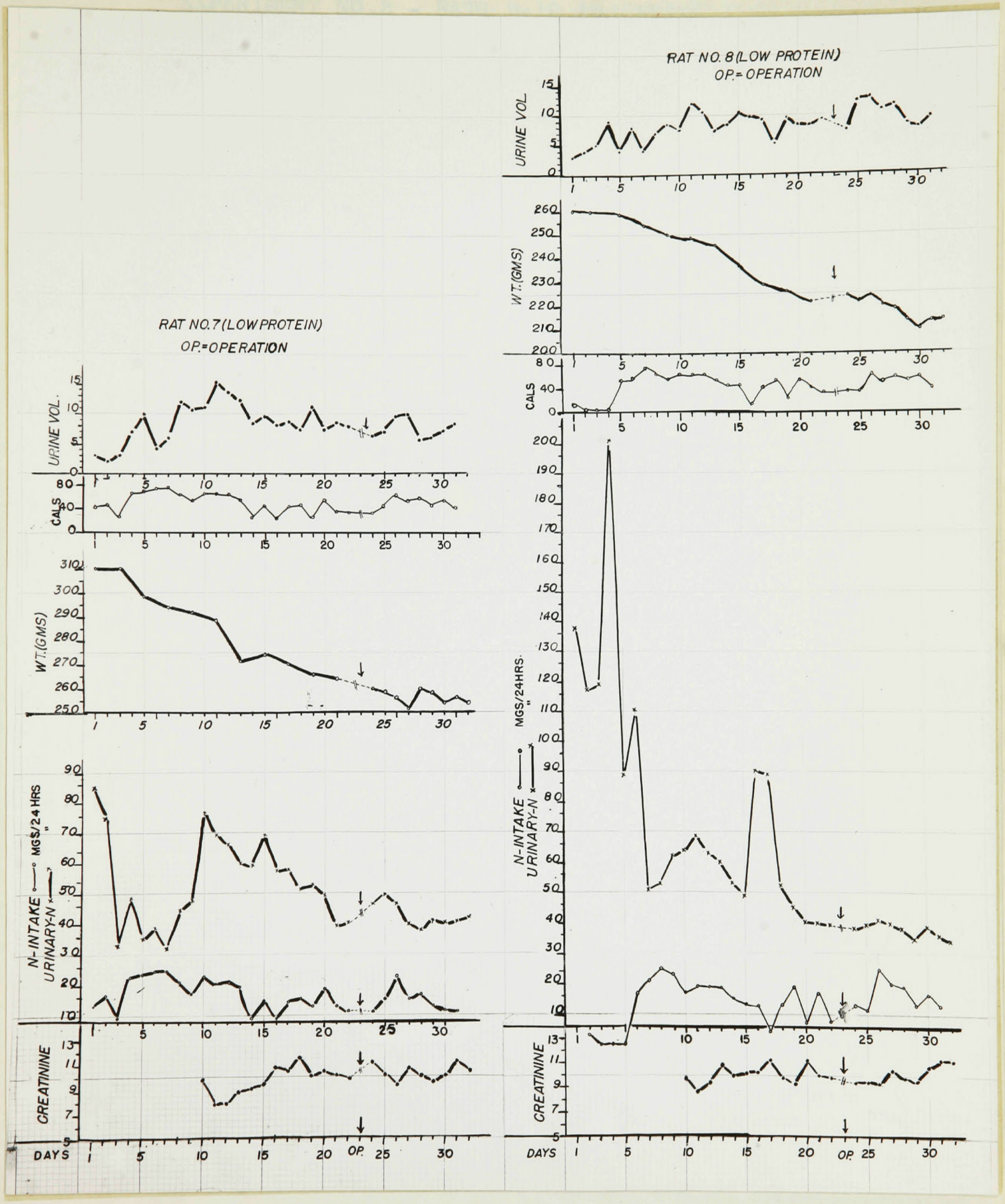

FIG. 5

Rats fed a low protein diet and damaged on 23rd day

EXPERIMENT NO.2 - RATS 9-12 inclusive

Assuming that the lack of adreno-cortical response to damage was responsible for the absence of a rise in urinary nitrogen output in rats fed the low protein diet, it was decided to see whether the administration of cortical extract would restore a metabolic picture similar to that seen in damaged rats fed Purina.

The method of collection and analysis of urine is the same in this and following experiments as that employed in Experiment 1.

The animals in this experiment were on the low protein diet for a period of 18 days prior to the commencement of metabolic studies, so that day 1 on the chart is actually day 19 on the diet. The reason that it was felt unnecessary to repeat the studies performed on rats 5-8 up to the period when a steady low level of urine nitrogen was reached. It also afforded the possibility of having rats always on hand to commence metabolic studies, (i.e., rats whose urinary levels had reached this low level without the necessity of their spending 18-20 days in metabolic cages for this level to be reached).

The rats chosen were adult male rats weighing initially 330-250 grams, as in all succeeding experiments, and it will be seen that this weight fell to approximately 260-280 grams prior to the commencement of metabolic studies.

EXPERIMENTAL RESULTS AND DISCUSSION

On day 1 of the metabolic studies (i.e., day 19 of low protein diet) rat 9 had reached the low level of urinary nitrogen, i.e., 40.4 mg. seen when rats 5-8 were operated on, but that in the next 24 hours it rose to 156 mg. and stayed at that level for

a day. Its caloric intake during this period was negligible, but on day 4 when it consumed approximately 35 cal. its nitrogen output fell to 85 mg. and stayed there till day 6. Then on day 6 and succeeding days when its caloric intake fell to 0 its nitrogen excretion rose to a level of 437 mg., a level seen on healthy adult rats fed a "Purine diet". It will be seen that the weight of rat 11 fell steadily and drastically.

This may be an example of the premortal rise Roche mentions. Whether this is purely the effect of the imposition of starvation (i.e., no caloric intake) or whether other complicating pathological factors are involved cannot be ascertained from the metabolic pattern of this rat alone, but the following experiments to be discussed will throw more light on the importance of the caloric intake.

It will be seen that in rats 11 and 12 the first day's nitrogen level falls to within the low range reached by rats 5-9 after 18 days of low protein diet, but that in rats 10 and 11 the failure of adequate food intake resulted in a rise in nitrogen excretion 24 hours afterward, so that in rat 10 the caloric intake for the first three days of metabolic studies was zero and the nitrogen excretion rose from 72 to 156 mg. and fluctuated thereabouts and on the fifth day fell to 41 mg. (i.e., day rat started to eat). When the caloric intake of the rat was maintained and sufficed (30 cal. per 100 mg. of rat) then the urinary nitrogen was steady, staying at the so-called "endogenous levels" and fluctuating around 40 mg. per 24 hours. On the 11th day the caloric intake fell to almost half of the rat's normal caloric requirements and in the next 24 hours the nitrogen excretion had

risen from 35-75 mg. The metabolic significance of this rise in nitrogen is obscured by the commencement of administration of cortical extract at this period. However, no such rise occurred with administration of cortical extract in rats 11 and 12. Also on the 18th day, one day after fracture of the left femur, (labelled operation in Fig.6) the caloric intake fell to zero and this was promptly followed in 24 hours by a rise in nitrogen excretion to a level of 93 mg.

Similar observations were recorded with rats 11 and 12. In rat 11 (Fig.7) it will be seen that during the 3 days of metabolic studies when the caloric was zero the nitrogen excretion rose from 54 mg. to 165; on day 4 when the caloric intake was 15 calories the nitrogen output fell to a level of 100 mg. Here the level of nitrogen output was lowered by an inadequate caloric intake on day 4, but not to the low levels seen when the rat's caloric intake was adequate. Then on the succeeding 3 days with a caloric intake of zero, the nitrogen output rose to 267 mg. and with consequent increase in caloric intake the nitrogen output fell to and fluctuated about a steady low level of 35-40 mg.

In rat 12 it will be seen that when the rat's appetite diminished on day 9 (for no apparent reason) a rise in the level of nitrogen excretion occurred 24 hours later and fell on subsequent days when caloric intake of rat was adequate.

To ascertain the effect of administration of cortical extract it was decided to try various levels in increasing amounts until that level was reached just below that which (when given subcutaneously) would increase the nitrogen output of rats which had previously reached the low level of nitrogen excretion in the

approximate range of 40 mg. per 24 hours, i.e., the level of cortical extract, which in the undamaged "depleted" rat would just not increase the nitrogen output above "endogenous level" of 40 mg. per 24 hours.

This later proved unpractical as levels up to 5 ml. of extract (in 1 cc. doses) were administered with no apparent effect on the nitrogen output, although Long et al (160) had shown that in intact adrenalectomized rats this amount would increase rate of nitrogen excretion (in young growing rats at least).

The subcutaneous route of administration was chosen as a precautionary measure insofar as Hartman, Lewis and Toby (236) have shown that after repeated intraperitoneal or intravenous injection of cortical extract into normal animals the Na retaining and K excreting effect disappeared and further Hartman, Lewis and McConnel (237) later reported that the above refractory state does not develop from subcutaneous injection, although this refractoriness with respect to nitrogen excretion was not mentioned.

The doses were increased from daily dose of 2 cc. (in four $\frac{1}{2}$ cc. portions) on the 11th day to 5 cc. (in 1 cc. portions) from the 15th day extending through until the 24th day. The dosage was divided because (a) this procedure facilitates subcutaneous injection and (b) is more effective in divided doses. Connaught Laboratory adrenal cortex extract was used throughout these and subsequent experiments.

The lack of any effect of this amount of cortical extract on the nitrogen output after trauma in these animals

is clearly seen in rats 11 and 12. With these animals the level of nitrogen output remains steady with little or no fluctuation for both the control period before and after damage. The "operation" is the same as that employed in Experiment 1, viz., open fracture of the left femur. In rat 10 any effect which the cortical extract might have had is obscured by the fall in caloric intake on day 18, with a subsequent rise in urinary nitrogen.

With the steady loss in weight there was an accompanying slow and steady fall in the level of the creatinine output so that the loss of muscle mass (presumably protein) in rats 10,11 and 12 may account for the fall in daily levels of creatinine excretions. It is also seen that the rate of loss of weight, after the fracture and during the period of cortical administration is not accelerated when compared with the preoperative period.

At the end of the experiment the three remaining rats 10, 11 and 12 were sacrificed and the adrenals removed, immersed in Suza's solution for 24 hours and then weighed.

T A B L E

Adrenal weights after prolonged feeding of low protein diet

Rat	L. ADRENAL (mg.)	R. ADRENAL (mg.)
9	52.3	52.8
10	19.8	19.0
11	21.2	21.6
12	18.7	19.1

It may be seen that in rat 9 the weights of the adrenals are significantly larger than those of either 10, 11 or 12, and have therefore undergone comparative hypertrophy in the face of starvation or some other pathological process.

In view of the fact that the diet fed was high in carbohydrate and low in protein content, to offset the possibility of fatty infiltration of the liver, choline in amounts which would more than suffice was included in the diet (238). Histological examination of the liver of rats 10, 11 and 12 revealed no marked fat infiltration.

It is seen that cortical extract in doses up to 5 cc. daily (divided doses of 1 cc. and subcutaneously) did not effect the urinary level of nitrogen excretion of a damaged rat which previously had been fed a low protein diet for 35 days.

It may well be that the steroids contained in this cortical extract are not necessarily those that are produced in excess in response to damage and accountable for the high levels of urinary nitrogen seen after damage to a healthy organism. Although investigations by Lukens and Dohan (239) have shown that for the adrenalectomized rat a daily dose of 5 cc. of cortical extract would replace the functional activity of the adrenal cortices by reinducing glycosuria of the intensity which characterized depancreatized animals having their adrenals intact.

That protein stores are not involved is further borne out by the observation that a rat fed a low protein diet until a steady low level of nitrogen excretion has been reached, withdrawal of food (voluntary or otherwise) results in an increased output of nitrogen 24 hours later. It should be remembered that when it is stated that an increased output occurs 24 hours later it is in actuality an increased nitrogen output occurring 48 hours after the onset of the decreased caloric intake. In some cases (as in rat 11) levels of daily nitrogen excretion were reached, during the fast,

which approximated the daily excretion of a 350 gram rat eating a diet (Purina) adequate in its protein content.

It is suggested that in the case of one "depleted" animal when faced with the prospect of deriving its caloric needs from its body tissue protein, the response on the part of the adrenals is such that an increased function on the part of the cortex occurs in an effort to meet the exigencies of an enhanced protein metabolism, somewhat similar to the mechanism suggested by Engel, Tepperman and Long (240).

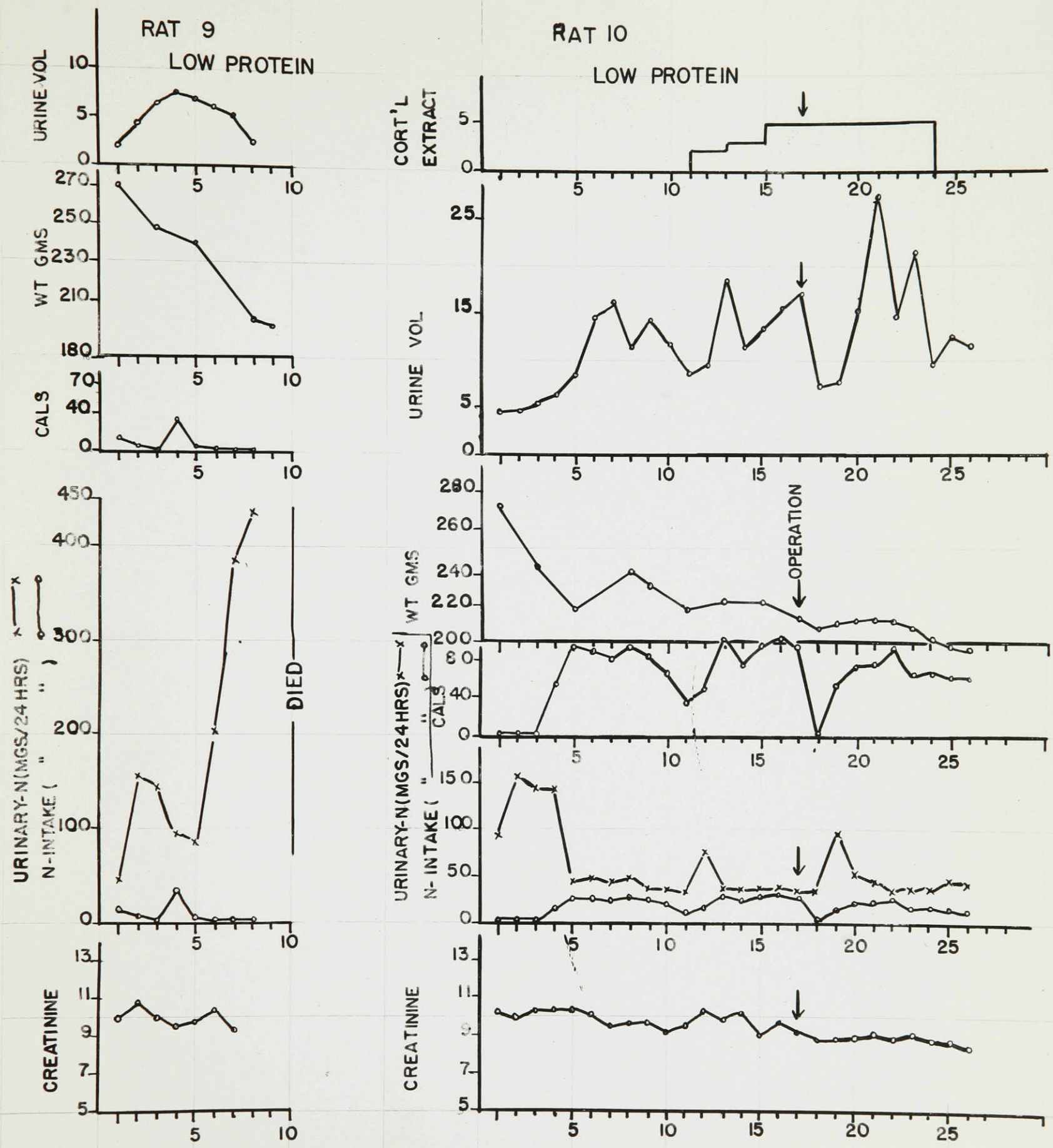

FIG. 6

Rat fed low protein diet, damaged, and in Rat #10 administration of cortical extract.

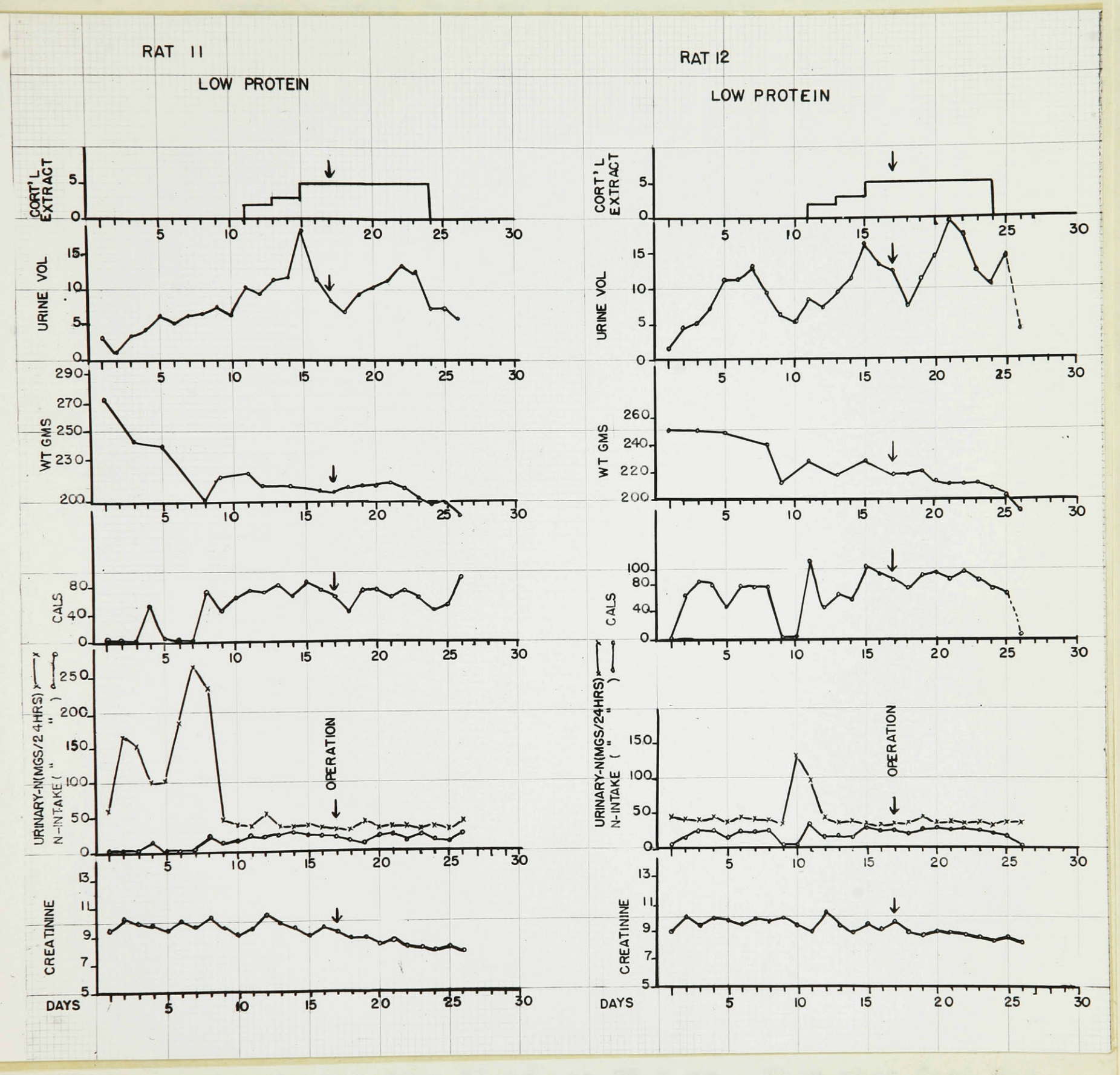

FIG. 7

Rats fed low protein diet, damaged, and administration of cortical extract.

EXPERIMENT NO. 3

RATS NO. 17, 18, 19.

EXPERIMENTAL RESULTS AND DISCUSSION

An endeavour was made in these experiments to elicit the response of increased nitrogen output by deliberately changing the caloric intake, in rats having reached "endogenous" urinary nitrogen levels and fed a low-protein diet.

The same strain of male hooded rats weighing originally in the vicinity of 350 grams were placed on the low protein diet for a period of 18 days before metabolic studies were commenced. As before at the outset, i.e., first few days with low caloric intake, the nitrogen excretions of the rats were comparatively high.

In rat 17, until the 9th day, the caloric intake was low and far from adequate and the animal drew upon its tissue protein as reflected in the high (but variable) levels of urinary nitrogen, ranging from 74.8 to 248.5 mg. then when adequate caloric intake was attained on the 8th day the nitrogen level fell to 30.3 but rose to 63.5 on the 11th day when caloric intake on day before had fallen to 8.4 cal.

In rat 18, after reaching a steady low level of nitrogen output on day 6, in the vicinity of 30-35 mg., a control period of 5 days was maintained after which on the 10th day the food was withdrawn for 2 days. In the two subsequent metabolic periods the nitrogen level rose from 31 mg. to 75.6 mg. Then when food was available and caloric intake was adequate on days 12-15, the daily nitrogen output fell to its previous levels of 32, 29.8, and 34.4 mg. per 24 hours in days 13, 14, and 15 respectively.

In rat 19, when on day 3 and for three succeeding days the caloric intake remained relatively constant the urinary nitrogen levels were maintained at 52, 32.5 and 29.8 mg. On 7th day rat became very ill and refused to eat for next 4 days, i.e., until it died. The nitrogen excretion rose steadily and reached level of 383.0 mg. on last day before it died, as is shown in chart (Fig.9) by dotted line.

So that in every case when rats maintained adequate caloric intake on a low protein diet after 18 days it maintained a steady and low level of nitrogen excretion, but a forced withdrawal of food or a self-imposed starvation elicited a marked and dramatic rise of urinary nitrogen output which manifested itself in the metabolic period 48 hours after the beginning of the fast.

It is interesting to note that in the rat Barbour, Chaikoff, MacLeod and Orr (241) found a mean of 0.16% of liver glycogen in rat fasted 24 hours and 0.32% for animals fasted for 48 hours. Ihis increased glycogen content of the liver after a 48 hour fast was found also by Cori (242). Cori's value for the 24 hour fasted rat was 0.10% and for the 48 hour fasted animal 0.397%.

More recently Cohn and Roe (243) employing standard conditions for dietary regime prior to the fast and for the method of obtaining the liver, etc., have found that marked glycogenolysis occurred during the 12-18 hour interval of fasting; at the 48-60 hour periods of fast the liver glycogen was measurably higher than during the preceding 24 hours.

It will be remembered that in the rats (henceforth termed "depleted rats) who had attained a steady low level of

urinary nitrogen (sometimes called endogenous nitrogen) a voluntary or imposed fast was followed by a rise in urinary nitrogen, 48 hours after the onset of the fast. This may well be correlated with the observed levels of liver glycogen at this time during the fast and it may well be that during the first 12 hours the rat is using more liver glycogen than is being laid down by gluconeogenesis and therefore sparing the latter, by the equilibrium lying on the side of the former; during the 12-18 hour interval the two processes are in true equilibrium and that from 18 hours on during the fast gluconeogenesis now is in the ascendance and this is reflected in the increased levels of urinary nitrogen during this period.

Returning to the question of labile protein stores it will be seen that they are either not involved, in view of the prompt heightened levels of nitrogen output during inadequate caloric intake, in the depleted rat, or this length of time of protein depletion does not exhaust such stores even though the level of urinary nitrogen has fallen to a steady low level.

It may be recalled that the work of Schoenheimer pointed to the concept of the biological organism as representing one great cycle of closely linked chemical reactions. The thermodynamic data demonstrate that hydrolysis and synthesis of protein cannot, under physiological conditions, be simply reversions of the same reactions. A "steady state" does exist in vivo, and this state is in the direction of synthesis, far from the thermodynamic equilibrium, in spite of an abundance of hydrolytic and oxidative enzymes demonstrably in operation even in the starving animal, the balance between them being maintained through the intervention

of other reactions. So that active resynthesis of protein occurs even in the starved animal.

Evidence has been presented in the section on protein stores that in the rat, at least, the serum proteins have been shown to fall more rapidly in low protein feeding than in starvation which again may suggest the possibility that reformation of protein may occur even when the animal is in negative nitrogen balance.

It may be here that the adrenal cortex fails to respond to the fracture but does to the starvation, though it is difficult to see why it should behave differently under these two conditions of stress. One wonders whether one can say that the existence of the animal is more threatened, in this depleted state, by a single day's fast than by an operation.

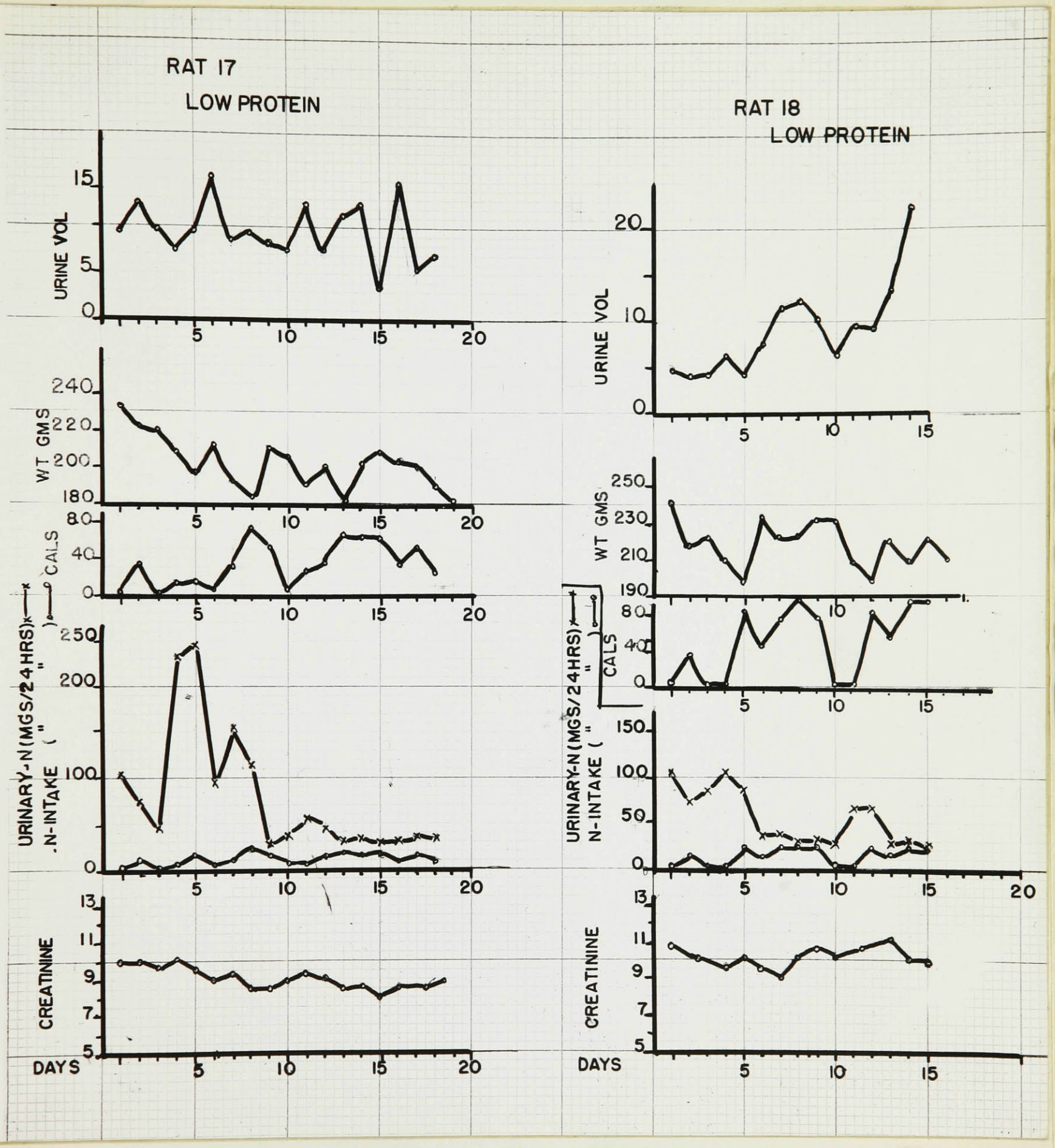

FIG. 8

Low protein feeding, and decreased caloric intake.

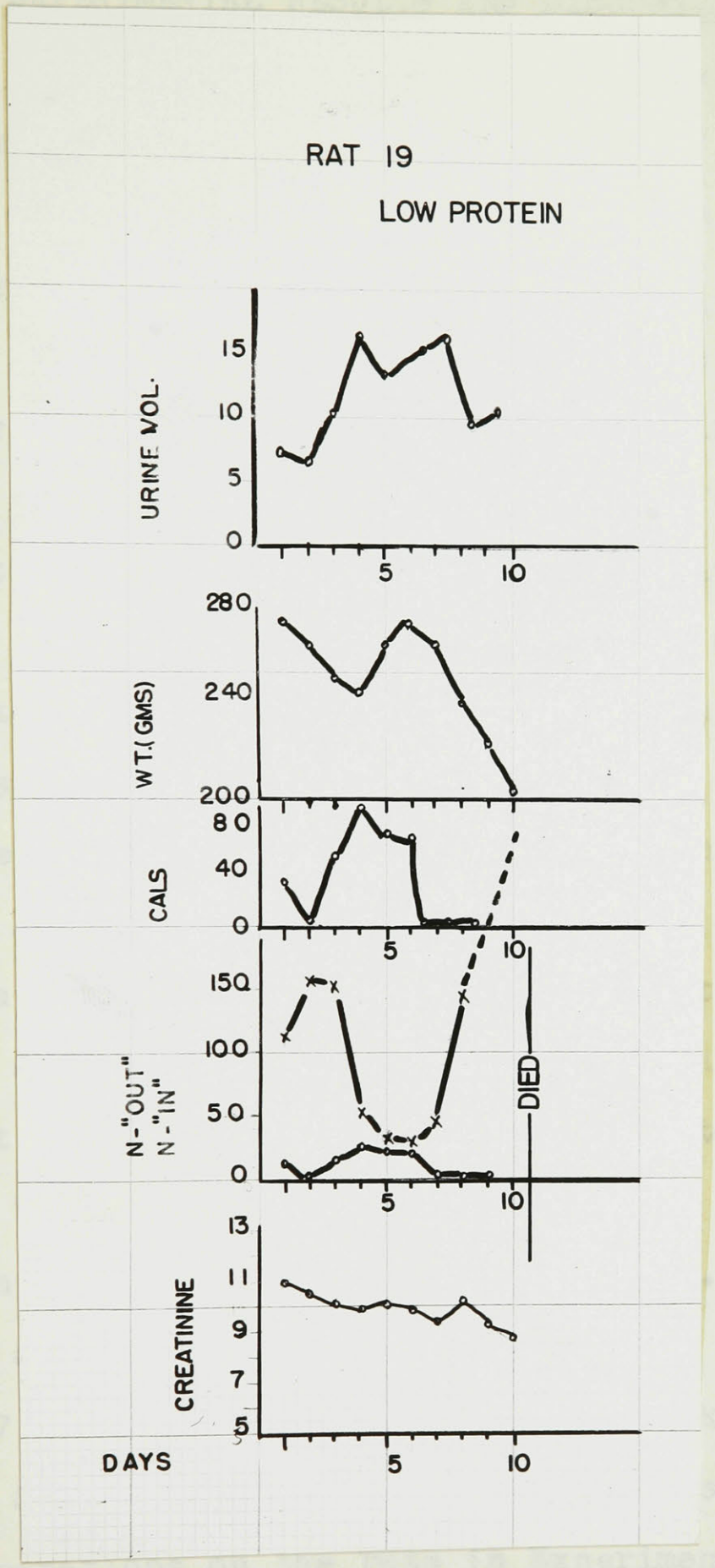

FIG. 9

Low protein feeding and pre-mortal rise in urinary nitrogen.

EXPERIMENT 4

RATS 21, 22, 23, 24, 37 and 38

EXPERIMENTAL RESULTS AND DISCUSSION

It w s decided to investigate whether the adrenals were essential in the "starvation response" (increased nitrogen excretion of "depleted" rats 48 hours after inadequate caloric intake) mentioned in Experiment 3.

As in Experiments 2 and 3, before metabolic studies were undertaken the male hooded rats, originally weighing 330-335 grams were kept on a low protein diet for a period of 18 days. Metabolic studies commenced on the 19th day, which is therefore day one on the charts. When the animals were placed in the metabolism cages their diet was changed to one identical in all respects to the former, but that in the salt mixture NaCL was substituted equimolecularly for the KCl.

A control period of 5-7 days was observed during which rhe rats attained a steady low level of nitrogen output and maintained an adequate caloric intake. The weight of the animals, except rat 2[illegible], falling at a slow steady rate during this period.

With rats 21-24 inclusive, 5 cc. of cortical extract were administered at day of each operation. Many unsuccessful bilateral adrenalectomies were done before the successful ones on rats 37 and 38 were performed, and thanks are extended to both Miss Stone for her helpful suggestions and to Miss Toby for the operations on the rats in Experiment 6. In rats 37 and 38 to ensure a better survival at time of second unilateral adrenalectomy the rats were primed in both cases with 5 cc. of cortical extract on the day prior to the second operation, during the day of operation, and in the case of rat 37 with an additional

5 cc. on the day after operation. In every case the cortical extract was administered in doses of 2½ cc. each.

It will be remembered from Experiment 2, that a daily dose of 5 cc. of cortical extract (given in 1 cc. doses) over a period of 5 days exerted no influence over the level of urinary nitrogen output in "depleted rats". In all rats on day of second operation the ordinary drinking water was replaced by normal saline.

In rats 21 and 22, (Fig.10) it will be seen that prior to the removal of the left adrenal, a steady low level of nitrogen output had been reached; the urinary nitrogen fluctuated in both cases around 30-40 gm., per 24 hours.

Rat 21 maintained an adequate caloric intake prior to and after the operation. No rise in nitrogen output occurred after removal of one adrenal alone, when adequate caloric intake was maintained, whereas in rat 22, its appetite failed on day of operation and so on day 7 the nitrogen excretion rose from previous level of 35 mg. to 82 mg. nitrogen per 24 hours, and fell the next day to its previous low level when rat's appetite returned.

In rat 23 it may be observed that an infection (on the back of its neck) noted on day 4 and persisting for 3 days, did not affect the levels of nitrogen output, the caloric intake being maintained at steady and adequate level during this period. Its left adrenal was removed on day eight, during which its caloric intake fell to zero. On day nine its nitrogen output rose from 32.1 to 61 mg. per 24 hours, and fell to 29 mg. on 10th day when its food intake was resumed.

In the foregoing discussion of the metabolic studies of rats 21-23 inclusive it will be well to consider that unilateral adrenalectomy serves as a control observation (a) whether after removal of the adrenals in the "depleted" rat, a fall in intake is followed by a prompt rise in nitrogen output and (b) unilateral adrenalectomy can be considered in the nature of a "sham operation".

From an examination of the charts of rat 24 (Fig.11) it will be seen that prior to adrenalectomy on the 5th day of metabolic studies a steady low level of nitrogen output was maintained. The left adrenal was removed on the 5th day and when the rat's caloric intake fell to the low and inadequate level of 11 cal. on the 6th day, a prompt rise in nitrogen excretion was manifested in the succeeding day which rose to a level of 63 mg. The right adrenal was removed on the 9th day and three days after food withdrawn for one day (on the 12th day); no rise in nitrogen excretion was seen to follow this lowered caloric intake in a bilaterally adrenalectomized animal, as was seen in intact and unilaterally adrenalectomized animals.

The lack of increased nitrogen output after food withdrawal in totally adrenalectomized "depleted" rats is more clearly seen in rats 37 and 38 (Fig.12). These rats were as all others, male hooded rats, who at the start of the experiment weighed 352 and 347 mg., respectively. They were unilaterally adrenalectomized at the beginning of the experiment and then fed the low protein and low potassium diet for 18 days, after which they were placed in individual metabolism cages. For a control period of 4 days they were allowed to reach nitrogen equilibrium, i.e., until the low and steady levels of nitrogen output were reached. The remaining left adrenal was removed in both on the 5th day -

on the 7th day food was removed from both. With a fast maintained until death (on the 9th day with rat 38 and the 11th day in rat 37) no rise in the levels of nitrogen output was noted as those seen before in intact or unilaterally adrenalectomized "depleted" animals, nor was a pre-mortal rise noted comparable to that seen in rats 9 and 19, nor was the rate of weight loss accentuated on the withdrawal of food, although a steady fall in the levels of creatinine output may be noted.

TABLE

Adrenal Weights

		Left mg.	Right mg.
RAT	21	18.6	19.0
	22	20.0	18.9
	23	19.3	20.0
	24	19.0	18.3
	37	43.2	23.4
	38	46.8	19.8

From Table it is seen that instead of compensatory hypertrophy of the right adrenals during the 18 day interval, a feature which Selye and Dosne (145) have shown to occur on a normal diet, a marked decrease in weight occurred. On histological examination the cortex was shown, in these animals, to have suffered a slight atrophy.

To eliminate the possibility of renal retention of nitrogen masking the response to fasting, blood N.P.N's, were determined on rats 24, 37 and 38 (on the 2nd day of enforced fasting in the two latter) the results as given below.

TABLE

		N.P.N. (mg.%)
RAT	24	27.8

		N.P.N. (mg.%)
RAT	37	31.2
	38	29.7

The determinations were done in duplicate, and the duplicates checked well.

Far from observing heightened N.P.N. values, they were on the average much lower than controls done in this laboratory which ranged from 35-41 mg.%.

Hematocrits were attempted with a limited amount of blood and were low. Great difficulty was encountered in taking blood from the tail, due possibly to the sluggish circulation of the adrenalectomized rat.

Before rats 24, 37 and 38 were maintained alive after complete adrenalectomy, 6 rats were lost. The metabolic studies done on them are not recorded here. In an attempt to combat the postoperative hypoglycemic shock, these rats had been given 10 cc. of a 10% glucose solution intraperitoneally, both on the day of operation and the following day. It may well be that this caused a crisis resulting in their death, as it has been frequently demonstrated that the adrenalectomized animal is extremely sensitive to intraperitoneal glucose injections (244,245,246,247).

CONCLUSIONS

From the above it may be concluded that the adrenal cortex is essential in the mechanism (S) underlying the rise of nitrogen output of a "depleted" rat to fasting or inadequate caloric intake (i.e., to meet daily metabolic requirements).

That renal retension does not mask an increased amount of nitrogenous substances is shown from an examination of blood N.P.N. values.

It is unknown as to how and from where the "depleted" totally adrenalectomized rat obtains caloric requirements during a fast, nor what those caloric requirements are.

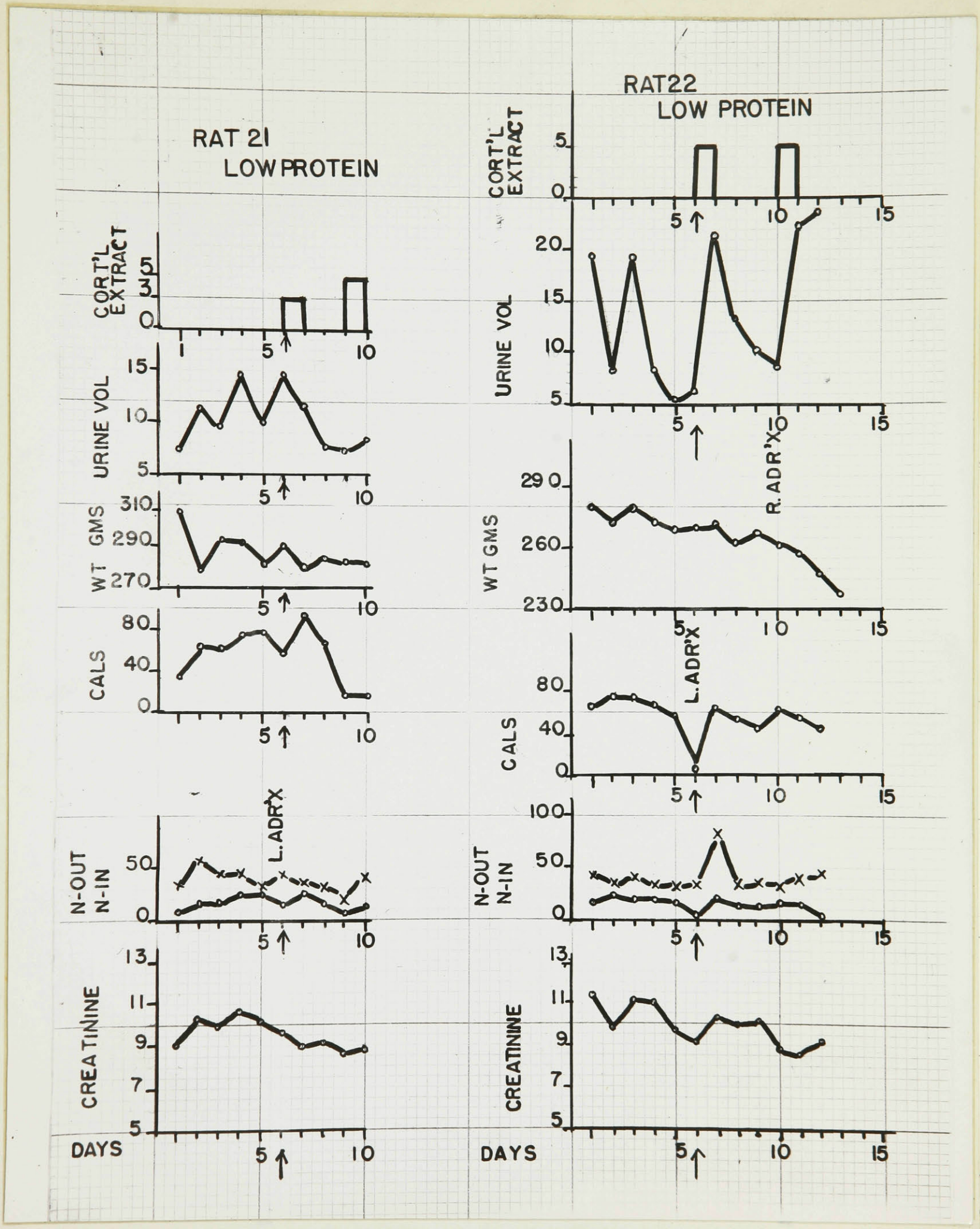

FIG. 10

Low protein feeding and total adrenalectomy.

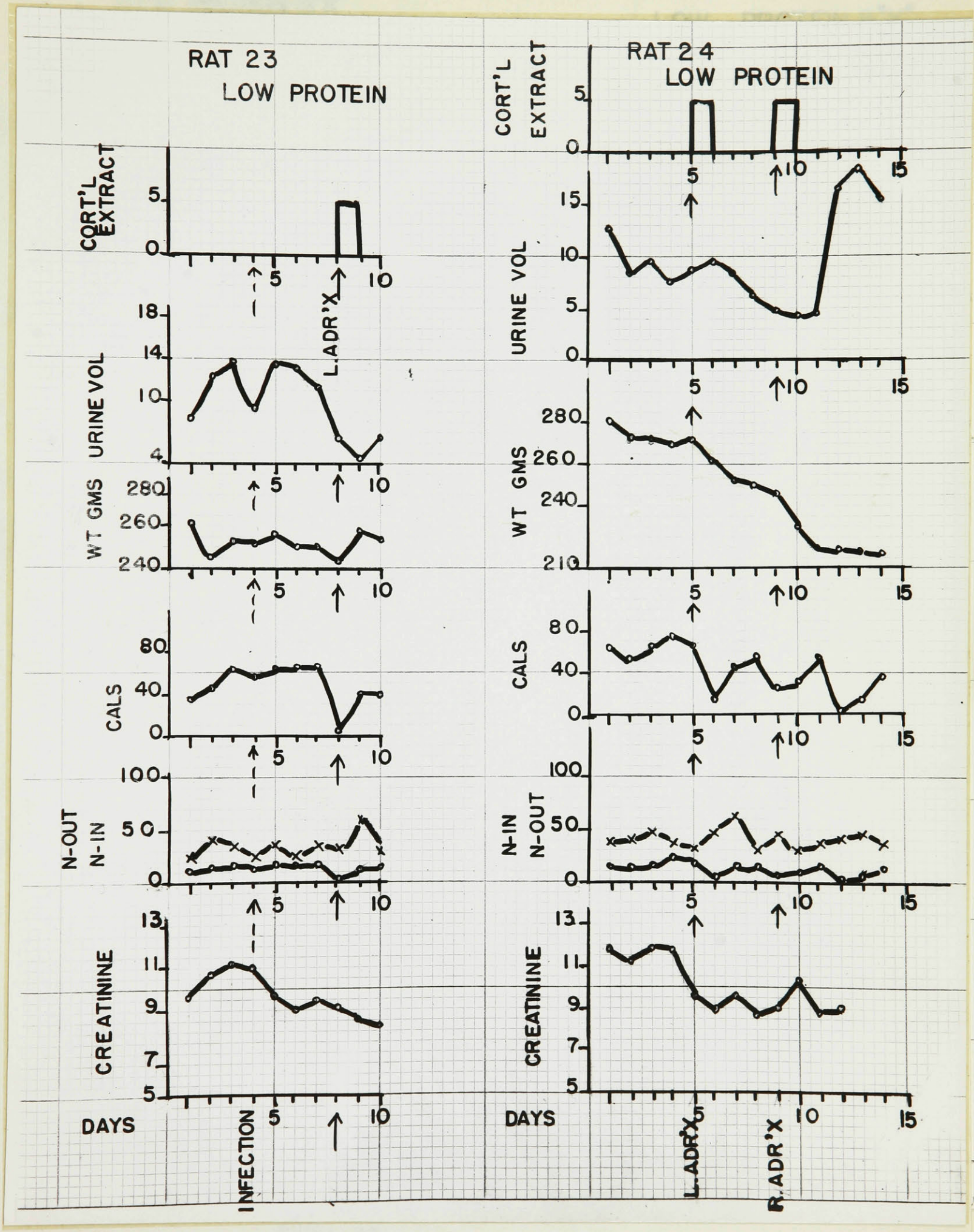

FIG. 11

Low prtoein feeding and total adrenalectomy.

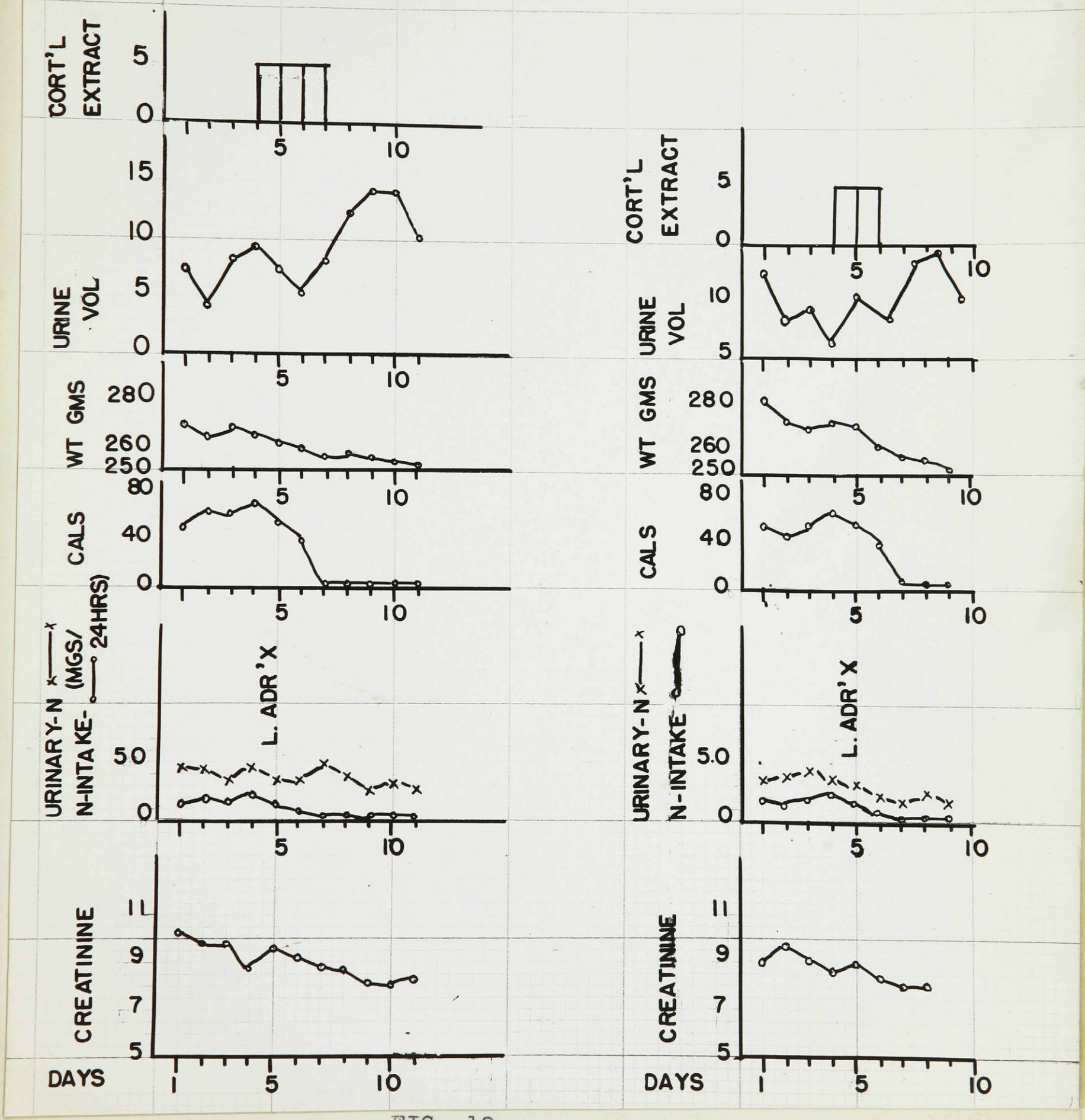

FIG. 12

Right adrenalectomy at start of studies; low protein feeding; then left adrenalectomy and zero caloric intake.

EXPERIMENT NO.5

RATS 25, 26 27, 29

EXPERIMENTAL RESULTS AND DISCUSSION

This is mentioned only in conjunction with the experiment following. The same conditions were employed here as in Experiment No.1, the animals being fed on Purina, except that the damage here was removal of the left adrenal under ether anaesthesia.

The resultant characteristic curve of nitrogen excretion repeats that noted in Experiment No.1. Here the peak of nitrogen output fell on the 3rd day after the operation in rats 27 and 29, and on the 4th day with rats 24 and 26. The net excess loss of nitrogen amounts to a somewhat smaller amount than with the rats where the form of damage was fracture of the left femur, the weight loss after damage being less in these animals.

From which we may conclude that when unilateral adrenalectomy is performed on previously healthy adult male rats (fed an adequate protein diet) a rise in curve of nitrogen excretion occurs, the peak of which varies with the individual animal, though usually falling on the 2nd to 4th day.

Turning to a consideration of the following experiment (6) then the above may be considered as a sham operation control with respect to total adrenalectomy.

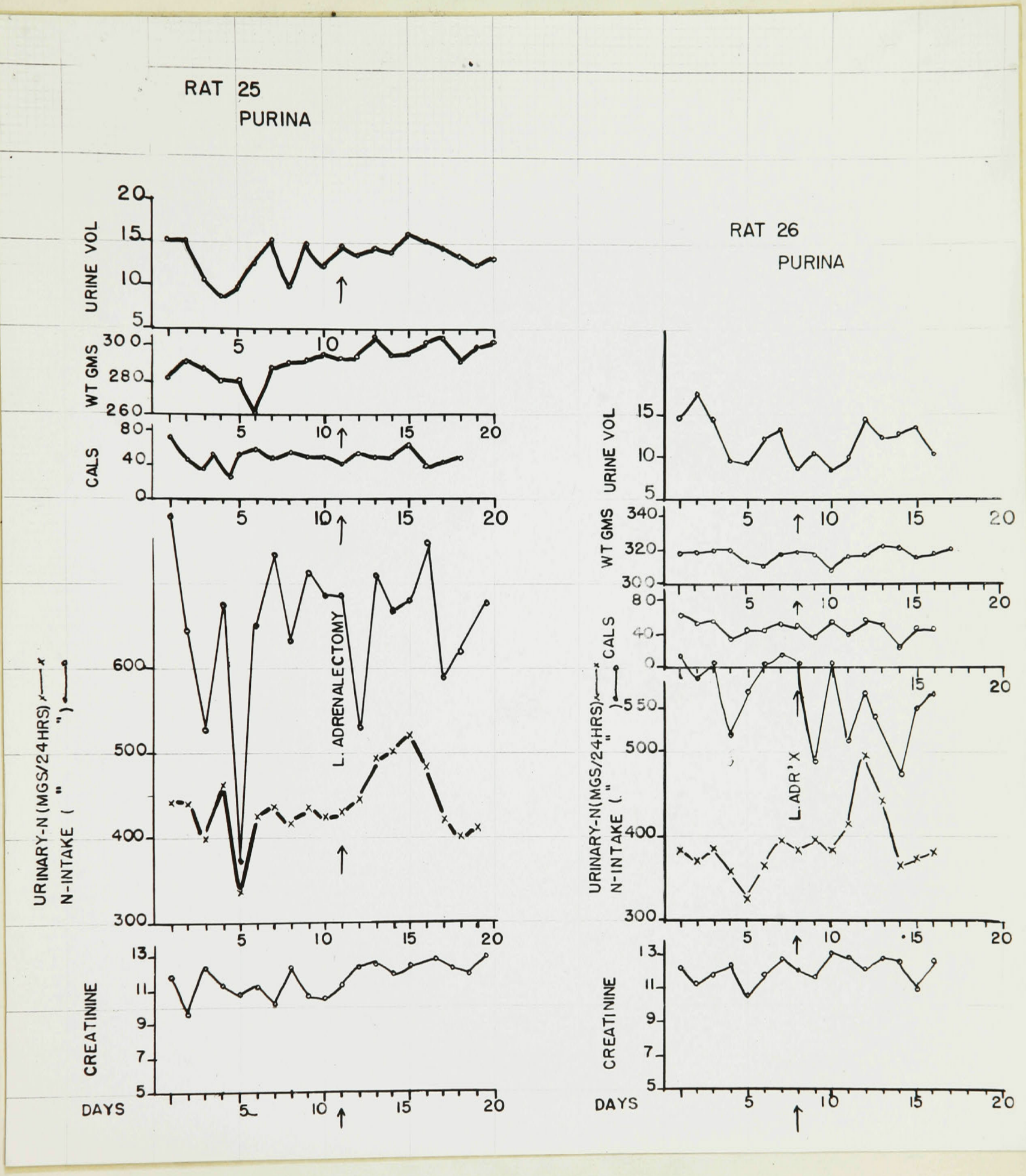

FIG. 13

Purina Feeding and unilateral adrenalectomy.

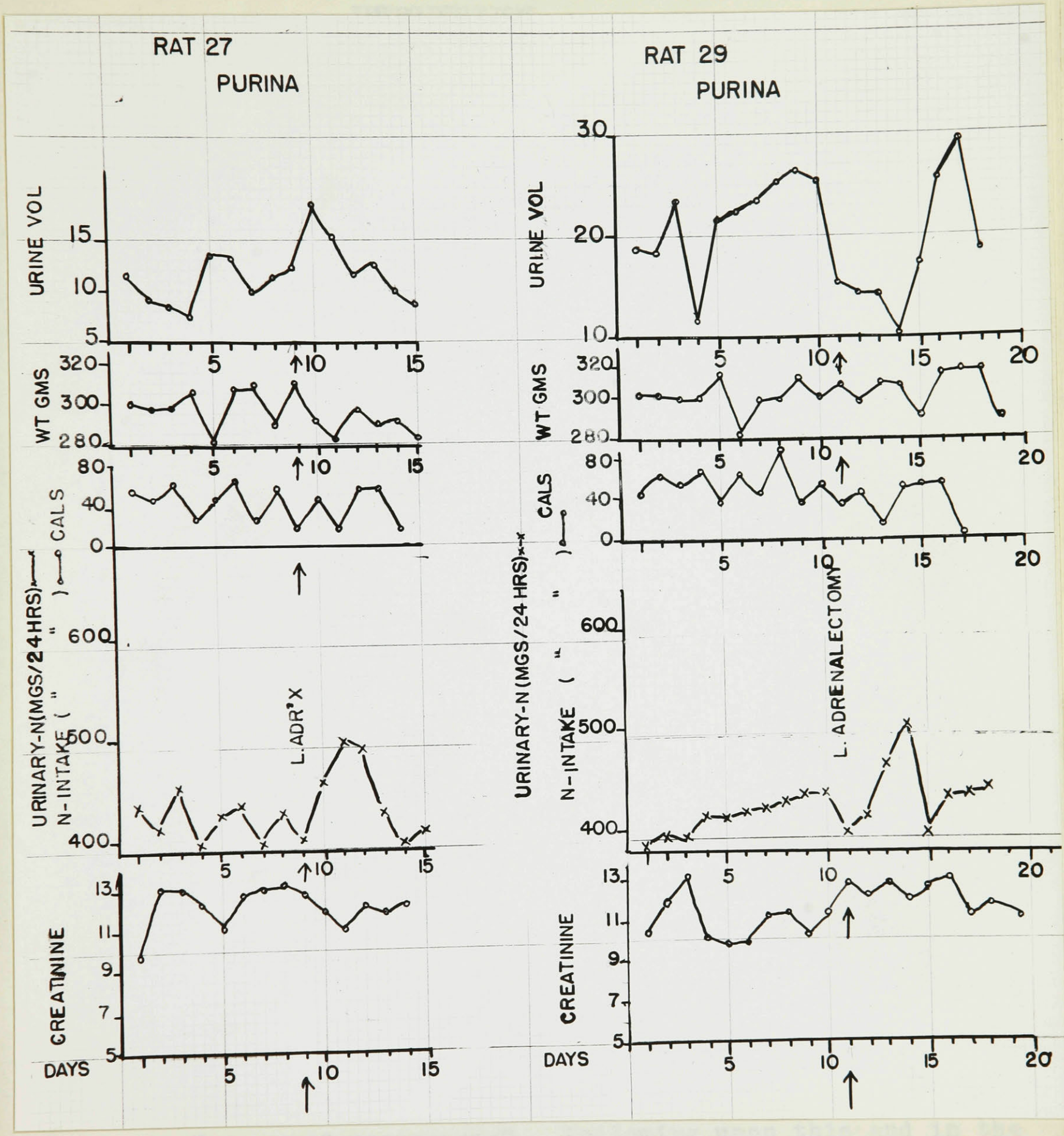

FIG. 14

Purina feeding and unilateral adrenalectomy.

EXPERIMENT NO.6

RATS 35-36 INCLUSIVE

INTRODUCTION

It has been shown that the loss of the adrenals renders the animal exceedingly sensitive to but minor amounts of trauma.

Selye (192) later observed that there was elicited a series of reactions on the part of the organism, which are common to all damaging stimuli to the body, and in a recent review (193) has summarized his findings. He states "In the course of our work on the pathologic and biochemical changes elicited by various noxious agents, we were struck by the fact that certain manifestations are always the same, irrespective of the specific nature of the damaging agent to which our experimental animals have been exposed". He has termed the sum total of features which characterizes the reaction of an animal to damage the "general adaptation syndrome".

This syndrome has been shown to consist of three general phases, an initial phase during which the animal's resistance decreases and then begins to increase, this period lasting for about 24-48 hours immediately following trauma and termed the stage of the "alarm reaction". Furthermore the stage of the "alarm reaction" has been further subdivided into a shock phase and counter shock phase. Then if damaging stimulus is continued the animal's resistance increased very markedly and there appears what has been termed the "stage of resistance". Following upon this and in the face of continued damage there occurs the "stage of exhaustion" in which the resistance of the animal eventually breaks down and it succumbs.

In the extensive research as to the underlying features of this "adaptation syndrome" various endocrine organs and

especially the adrenal cortex have been shown to occupy a prominent part (249,250,251,252).

Selye and co-workers have found that at the end of the counter shock phase, the adrenal gland is found to have discharged its lipid granules and this is taken to be a sign of increased activity, since the lipid granules are presumed to be carriers of the fat soluble hormones. It is during this counter shock phase that Browne and co-workers (184,185) have shown a rise in the corticoid activity of the urine.

The adrenal gland also shows characteristic changes during the three stages of the "general adaptation syndrome". They are greatly enlarged during the later stages of the "alarm reaction", approach normal during the stage of "resistance" and undergo a secondary enlargement during the stage of "exhaustion".

That the thymus and lymph nodes occupy some role in this syndrome and that a reciprocal relationship exists between these and the adrenal cortex is borne out by the following observations.

The thymus and lymph nodes have been observed to undergo pronounced atrophy during the "counter shock phase" at a time when the adrenal cortex shows the most pronounced signs of hyperactivity. Cortical hormone injections also cause marked thymus atrophy while adrenalectomy prevents the involution of the lymphatic organs during the "alarm reaction". Further it has been shown that there exists a similarity of response of the thymus and lymph nodes to the administration of adrenocorticotrophic hormone in the rat at least, and this to consist of a general involution (253,254).

In an attempt to summarize these findings the chart below, reproduced from Selye's review is shown.

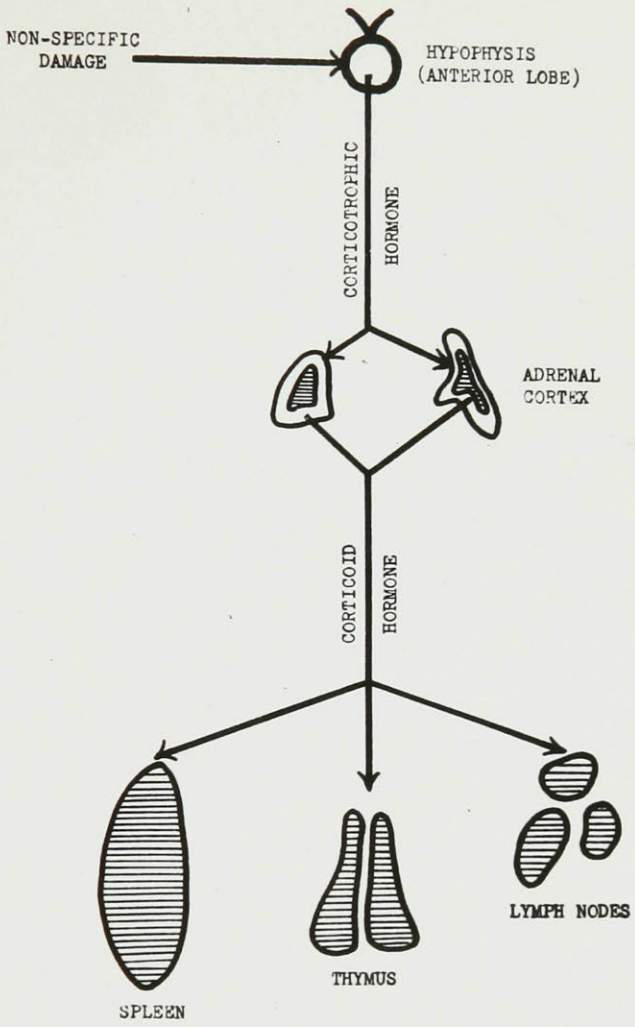

Diagram Illustrating Interrelations Between Hypophysis, Adrenals and Lymphatic Organs.

FIG. 17

In explaining the chart Selye concludes "Nonspecific damage stimulates the pituitary (pathway unknown) to produce an increased amount of corticotrophic hormones. This, in turn, stimulates the adrenal cortex to enlarge and to secrete excess quantities of corticoid hormones. The latter increase resistance and at the same time cause involution of the lymphatic organs".

Each step in the above chain has been proven by extirpation and by injection experiments.

Bearing upon all these findings is the possible relationship between the above "adaptation syndrome" and the already mentioned N and S hypothesis, as in both the adrenal cortex plays an integral role in the response of the animal to nonspecific damage.

In the former it serves as the agent which accounts for increased resistance to trauma, and in the latter, by an imbalance in its secretion after trauma, plays an important role in the causation of the heightened nitrogen output seen after damage.

The experiments about to be described below were drawn up in order to investigate the role that the adrenal might play in the change in nitrogen metabolism after trauma in animals fed an inadequate diet.

EXPERIMENTAL RESULTS AND DISCUSSION

Rats, of the same strain and weight range employed in previous experiments, were totally adrenalectomized after a 4 day control period study. A diet of approximately the same protein content as Purina, but low in potassium was fed these animals during the entire period of metabolic studies.

On the 10th day of studies (6 days after total adrenalectomy) the rats were all subjected to the same form of damage used throughout these experiments - that of open fracture of the left femur. On the day of this operation 5 cc. of adreno-cortical extract were administered to each rat. Macroscopic autopsy after death revealed no accessory adrenal tissue in any of the animals.

It will be remembered from Experiment 5 that after unilateral adrenalectomy there occurred a rise in the curve of nitrogen excretion similar to that seen in the rats who suffered a fractured femur, and furthermore that in humans this increased nitrogen output

following damage of all sorts (termed "N Catabolic Process") and was a characteristic feature of the post-traumatic metabolic process.

From a close scrutiny of the metabolic charts on rats 33-36 inclusive, it will be seen that this process as reflected in the nitrogen output is apparently lacking.

In rats 33-35 the nitrogen output fell from a level of about 400 mg. daily in the precontrol period to approximately 300 gm. dailt after total adrenalectomy. In rat 36 the drop in nitrogen excretion was even greater and fell to a 200 mg. level. That this was not due to impaired renal clearance is born out by the diuresis encountered during this period and also by the fact that the daily creatinine output was fairly constant throughout.

That various factors may have contributed to this lowered level of nitrogen excretion is probably true. The intricate endocrine and metabolic relationships which directly or indirectly involve the adrenal are in all probability not fully known as yet. Certain observations though which bear on this problem should be kept in mind.

Carr and Beck (255) showed that after bilateral adrenalectomy in the rat there is a reduction of approximately 25% in the total metabolism. Ashworth and Cowgill (256) in the study of the adrenalectomized rat (weighing 100 grams) found a diminution of 40% in its overall metabolism.

Recently Toerkischer and Wertheimer (9) have reported findings which indicate that 24-28 hours after total adrenalectomy of the rat there occurs a sharp decline in free and total acid in the stomach and a precipitous fall in the proteolytic enzyme content of the intestines.

So that superimposed on a decreased metabolism is a possible failing of digestion and absorption of protein in the adrenalectomized rat. It would be interesting to do both feces and urinary analysis on the adrenalectomized animal.

Further the work of Long et al (257) has shown that one of the influencesof the cortex is in the formation of carbohydrates from amino acids or their residues after deamination, so that decreased gluconeogenesis might also be a contributing factor in affecting the lower levels of urinary nitrogen output after adrenalectomy in the rat.

It has been shown that fasted adrenalectomized mice and rats excrete less urine nitrogen (160) and also that adrenalectomized rats and mice oxidize glucose at a more rapid rate and are more wasteful in the handling of their glycogen stores than intact animals.

In rat 33 a fall in caloric intake on day 6 was followed by a noticeable increase in urinary nitrogen on the following day.

After adrenalectomy of each animal there occurred a steady slow loss in weight until death.

A period of 6 days of metabolic studies was done before the adrenalectomized rats were subject to fracture. In rats 23-36 in the 4 days following this (labelled damage on chart) there did not occur any increase in N output but actually a slow steady fall. Only in rat 33 on 2nd day after damage was there a slight rise in nitrogen output and this was in no way comparable, both with regards to amount and duration, to that seen after damage of a previously intact animal. Even the ingestion of a semi-adequate caloric intake during the period after damage caused no increase in nitrogen output to that over the pre-damage period.

It will be interesting to recall that Putschkow and Krasnow (258) in their studies in adrenalectomized cats and dogs, found a decrease in urea nitrogen in the urine, which they attributed to an impairment of liver function. Whereas in a study of nitrogen and sulfur metabolism of adrenalectomized (3 months old) rats Sandberg and Perla (259) noted that immediately following adrenalecotmy there occurred an increase in nitrogen excretion. Nitrogen retention decreased from 52% of the intake during the control period prior to total adrenalectomy to an average of 33% for 9 days after adrenalectomy, then to 39% and 30% in succeeding two periods consisting of 17 and 5 days respectively. The food intake for these periods was somewhat lowered but not sufficiently to account for the drop in retention. Another striking feature is the pronounced creatinuria encountered in the period immediately following adrenalectomy. Both this and the increase in the excretion of total urinary sulfur as well as that of urinary sulfur resemble the observations of Cuthbertson (120). During the whole period Sandberg and Perla report that the fecal nitrogen excretion remained unchanged which is somewhat surprising in view of the report of Toerkirscher and Wertheimer.

The accompanying chart (Fig.18) which has been drawn from the tabulated results of Sandberg and Perla shows that in the period following adrenalectomy (first 9 days) there occurs an increased nitrogen excretion which is followed later by a still further rise in the level of nitrogen output, the plotted results being averages. An interesting feature is that throughout the studies the animals are in positive nitrogen balance. In view of the fact that daily studies are not reported it is difficult to evaluate their results, further the tendency for an increased nitrogen excretion to persist a month after the operation suggests that one is viewing

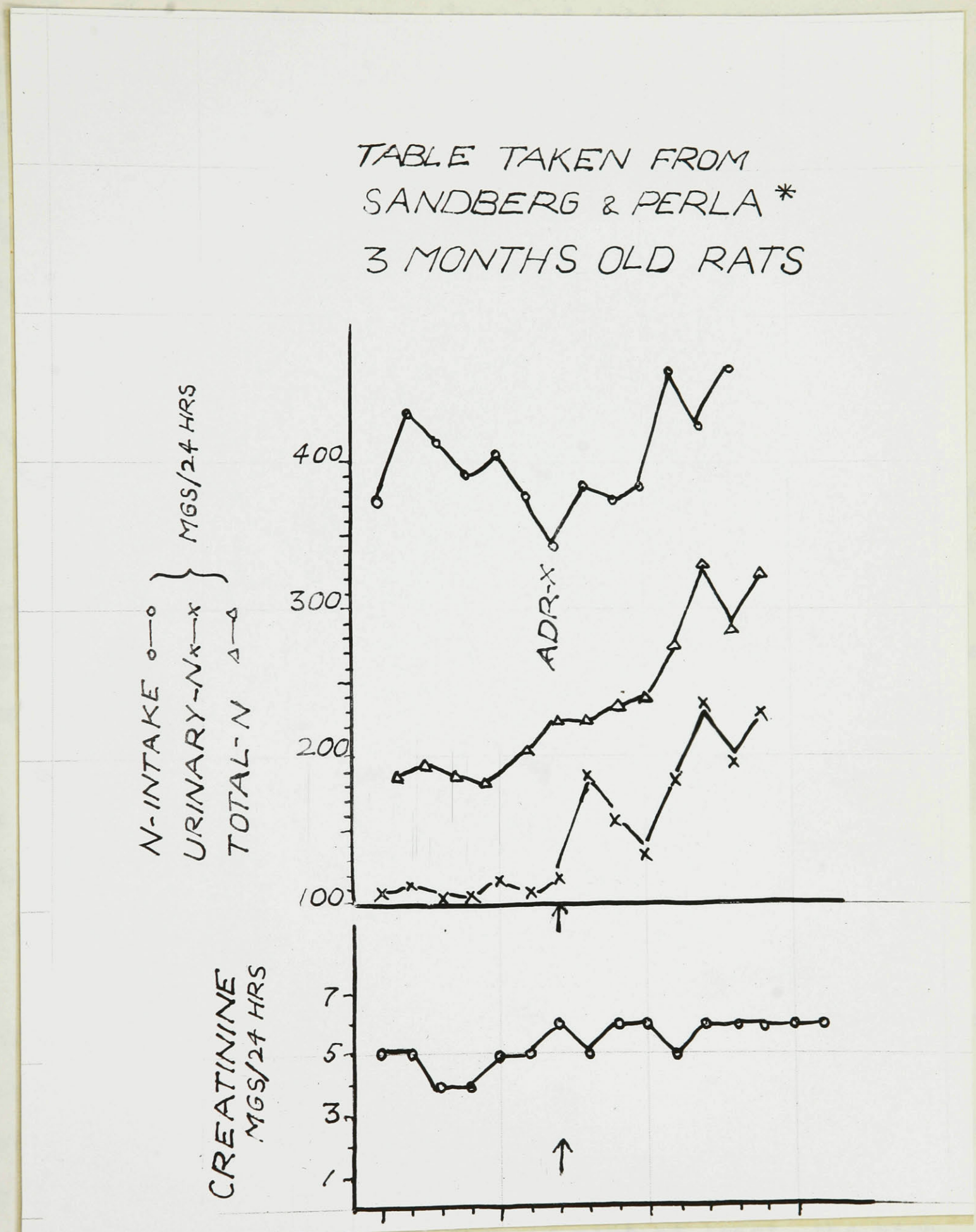

FIG. 18

Growing rats adrenalectomized. Drawn from results of Sandberg and Perla. Values are means of their

the effect of increased growth rather than the "protein catabolic oricess". They report that an increased intake accompanies the increased nitrogen output throughout this period after the operation (during which the rats still continue to grow).

From the above we may note that, whereas after unilateral adrenalectomy in adult male rats, fed an adequate protein but low K diet, a characteristic rise in nitrogen excretion occurs similar to that seen after fracture of the left femur, in totally adrenalectomized rats no such rise occurs. Renal retention does not play any role here.

That whereas Sandberg and Perla have reported an increase in nitrogen output (confined to the urea fraction) after total adrenalectomy in growing rats, the continued growth after the operation obscures the true metabolic process, and suggests incomplete adrenalectomy.

It is suggested that in view of the findings reported here (Figs. 15 and 16) the adrenal cortex might well occupy a central role in the response to nonspecific damage reflected in the heightened post-traumatic levels of urinary nitrogen.

A further damaging stimulus to an already adrenalectomized animal does not elicit the metabolic pattern (i.e, characteristic curve of increased nitrogen excretion) as seen in previously intact animals which further strengthens the conviction that an increased output of glyconeogenic substances by the cortex in response to damage accounts for, in part at least, the high levels of urine nitrogen seen in the period termed the "Protein Catabolic Process".

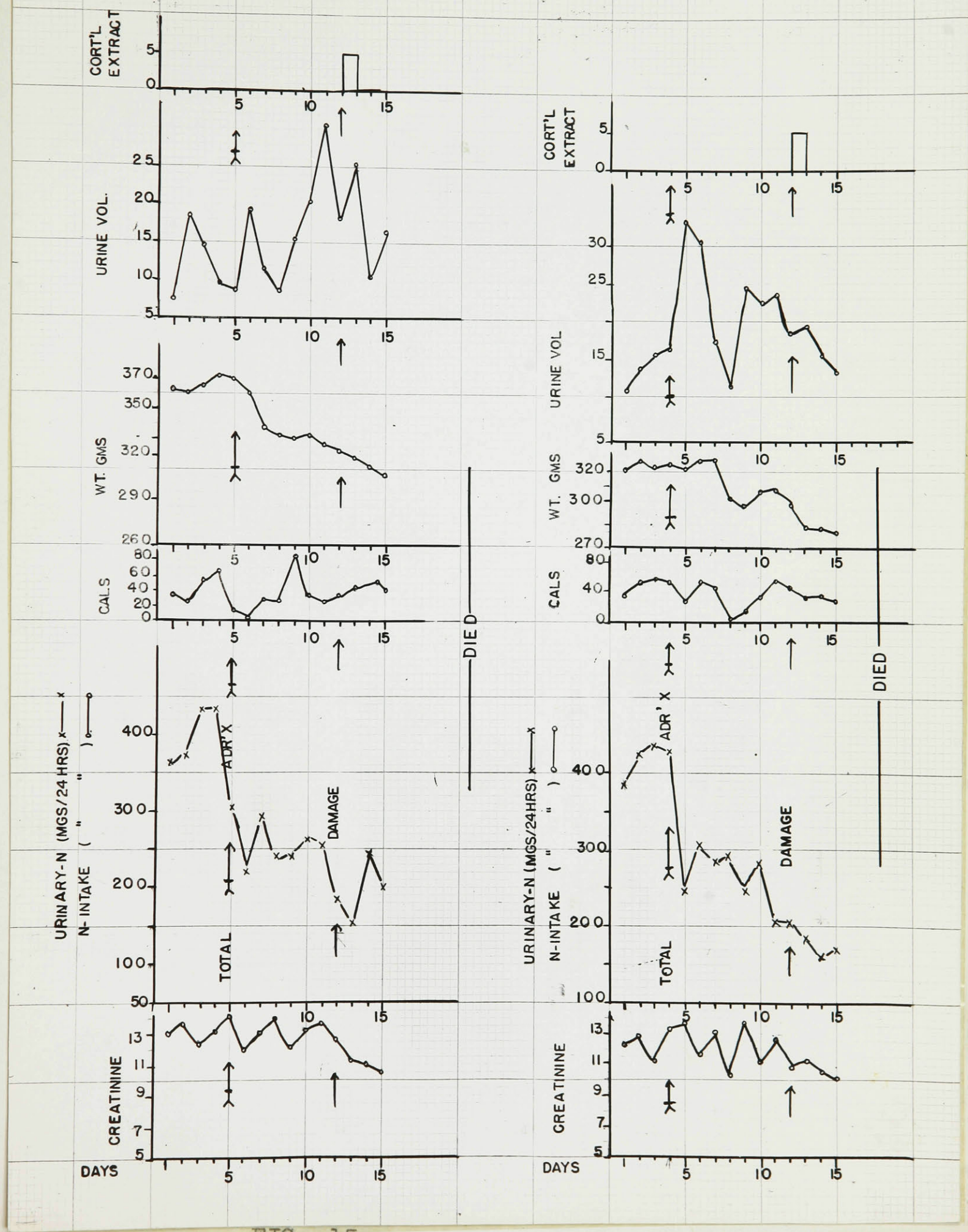

FIG. 15

20% protein and low potassium diet;

bilateral adrenalectomy.

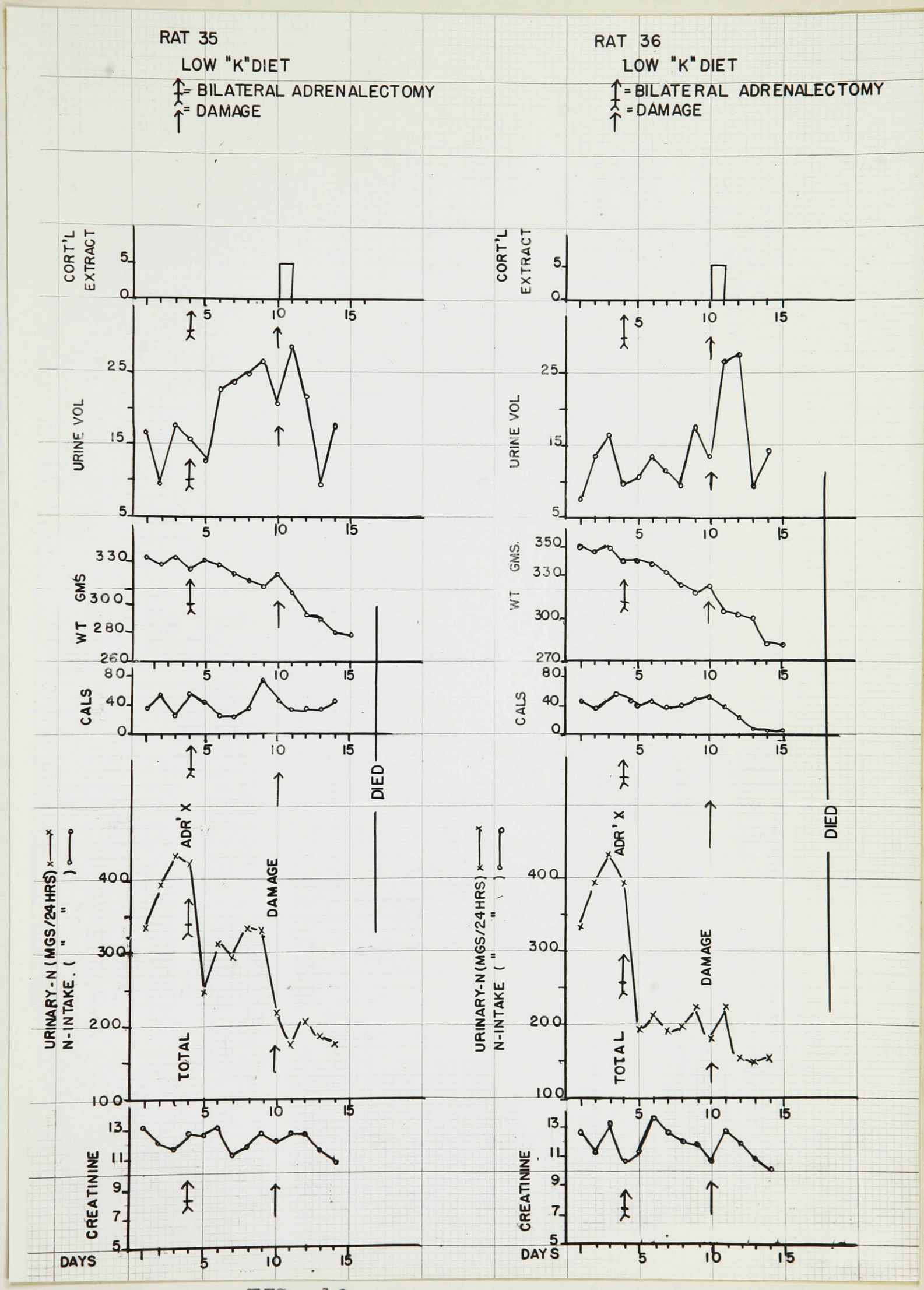

FIG. 16

20% protein and low potassium diet;

bilateral adrenalectomy.

GENERAL DISCUSSION

To present a unified hypothesis as to the nature and mechanism of the role that the cortex plays in normal bodily economy and in response to damage is, of course, impossible, as many unexplainable observations exist and probably many important features have not as yet been discovered.

Further, the many intricate relationships between the adrenals and other endocrines apart from its direct modifying actions on many organs, will have to be clarified before a clear perspective as to the exact nature of the role of the adrenal in the body's economy is attained.

In any event many excellent reviews have been published that deal with the overall function of the cortex which Ingles (260) and the more recent one of Swingle's (261) are to be noted.

Taken separately many functions of the adrenal have been postulated of which the following are some:

1. Sodium metabolism and renal function, (Loeb) and (Harrop).
2. K metabolism (Kendall) and (Zwemer).
3. Electrolyte and water metabolism, (Swingle).
4. Capillary tonus, (Swingle).
5. CHO metabolism, (Britton) and (Long).
6. Phosphorylation processes, (Verzar).
7. Renal formation of ammonia, (Jimenez-Diaz).
8. Function of sympathetic nervous system,(Stecker).
9. Adaptation, (Selye).
10. Shock, (Moon).
11. N metabolism after damage and "N and S" hypothesis, (Albright and Browne).

That the cortex is concerned with all of the functions mentioned and probably more, there can be no doubt. The question is where the emphasis is to be laid, if at all, or whether to view these more in the nature of a dynamic integrated whole where the impairment of cortical function affects all these together, and functions of each in turn bearing a dynamic relationship to the others. The work of many investigators, including the pioneering work of the Long's school has shown that there indeed exists an intimate and delicately balanced relationship between the adrenal cortex and the metabolism of protein, especially with regard to gluconeogenesis.

Further extending results of the above studies, Browne and Albright have postulated that the cortex secretes normally at least two hormones, one termed the "S" hormone which is anti-anabolic with respect to protein, and the other "N" hormone which is concerned with the anabolism of protein and that whereas in the normal healthy organism these are delicately balanced, after damage an imbalance occurs, in the initial phase of which the balance swings over in favor of the "S" hormone, later to be followed by a swing in the other direction.

Studies on the excretion of gluconeogenic substances (termed urinary corticoids) excreted after damage tend to bear this view out.

Investigations of urinary metabolites after damage have revealed that there occurs an increase in N, S, and P output accompanied by a marked creatinurea and further that the S:N and P_2O5:N ratios suggest that the material catabolized during this period was mainly muscle protein. The significance of this is as yet in doubt.

The work of Cuthbertson (78) and of Whipple (75,102) on nitrogen balance studies in animals (rat, dog), who have been drained of their protein either by dietary and plasmapheretic approaches, has led these investigators to believe that the source of the increased urinary nitrogen output seen after damage to the healthy organism, lies in what one calls "storage" and the other "reserve" protein. It has been shown that even in those animals which have been on deficient protein diets for a period sufficient to bring nitrogen excretion to a low level and which behave after fracture as did the animals in Cuthbertson's experiment (78), in which he claimed had exhausted their storage protein, a prompt rise of nitrogen output occurred on reducing the caloric intake. This failed to occur in similarly treated animals after adrenalectomy. And further in the case of two depleted animals concomitant with a pre-mortal rise in urinary nitrogen excretion was a comparative hypertrophy of their adrenals.

If the failure to show a rise of urine nitrogen after trauma, with the depleted organism, were a result of a depletion of a reserve of protein which could be catabolized, then the supplying of the organism with amino acids and peptides - the products of such a breakdown should be followed by the appearance, in the urine, of increased nitrogen, whereas it has been shown (221) that "amigen" is retained in "depleted" individuals, as is food protein.

If after injury the state of the body's previous protein reserve was the determining role in the degree of heightened nitrogen output, then one should be able to reduce the negative nitrogen balance by exogenous intake. Some have claimed to have achieved this (263), but it has been pointed out that when the balance was achieved during injury that it was accomplished in

patients who were "protein depleted" before the injury and as has been shown in the experiments reported here and elsewhere (103,221) these patients have shown an abnormal tendency to cling to exogenous protein. Madden and Clay (103) have maintained that it is the depletion of protein stores rather than the lack of "inhibition of anabolism" which is responsible for the lack of the manifestation of a "Protein Catabolic Process" after damage in the "depleted animals" and have said " that if inhibition of anabolism" were the important factor one should expect it after damage in the depleted as well as the normal. Remembering that the depleted organism has been shown to abnormally cling to introduced protein even after damage, it might seem that this was the case were it not for the fact that it has been shown here in rats and elsewhere in humans (219) that faced with the prospect of a fast, or in the face of near death, both have shown an increase in nitrogen output almost equal to that seen after damage of a previously healthy organism and further in the "depleted" human case studied in this laboratory (219) a large pre-mortal increase in the excretion of gluconeogenic substances occurred, whereas in the case of rats, their adrenals, under these circumstances, underwent comparative hypertrophy.

More likely it is the "inhibition of anabolism" that is the factor preventing the attainment of nitrogen balance after damage as it has been shown by others that nitrogen intake during acute injury merely increases output (103).

In an elucidation of the above a possible experimental approach would be that of the evaluation of the state of the body protein prior to an injury - such a test as yet unfortunately has not been reported in the literature.

It has been pointed out that the significance, either from a clinical or biochemical viewpoint, of the negative nitrogen balance seen after injury of previously healthy individuals is still unknown. Some have suggested that it is a breakdown of protein to provide specific fractions for combatting the injury. It has been recently reported that the amino acid methionine may largely prevent urinary nitrogen increases after burns in rats (141) although similar experiments with burned humans in this laboratory failed to confirm these observations.

On the basis of adrenal extirpation experiments it has been shown that the heightened nitrogen output seen in damaged rats is absent here and that probably the cortex occupies therefore an essential role in the mechanism of the "Protein Catabolic Process", although here various complicating factors would tend to observe a clear cut deduction - namely low free gastric HCl and intestinal proteolytic enzymes and lowered overall metabolism of the adrenalectomized rat. Taken together though, they do not account for the absence of increased rise of urinary nitrogen after total adrenalectomy in the face of inadequate caloric intake and therefore it would seem that a more direct function of the cortex per se is involved, as a lowered intake or absorption cannot account for a failure to rise after trauma, since it was shown decreasing the intake actually caused a rise in nitrogen in the intact animal.

Lastly the possibility that after trauma in the previously healthy organism the increased nitrogen output might come from the thymus and lymphoid tissue is suggested, in view of the previously mentioned findings of White and Dougherty (253,254).

S U M M A R Y

(1) The literature was reviewed concerning certain metabolic and physiological phases of the response to damage in man and rat, protein metabolism, and of the functional relationship of the adrenal cortex in the resistance and response to damage.

(2) In contrast to the previously healthy and adequately fed adult "hooded" male rats, those fed an almost protein-free diet until a steady low level of nitrogen was maintained showed no increase in urinary nitrogen output after fracture of the left femur or unilateral adrenalectomy. This confirms the findings of Munro and Cuthbertson.

(3) Adrenal cortical extract in daily doses up to 5 cc. (in 1 cc. doses) failed to restore the urinary nitrogen pattern in "depleted" rats after damage to that seen in previously healthy animals when subjected to similar trauma.

(4) It was further shown that a rise in the daily levels of nitrogen excretion was consistently elicited in "depleted" rats 48 hours after a reduction in caloric intake to below a certain level making it seem doubtful that a depletion of labile protein stores accounted for the failure to show a rise in urinary nitrogen after fractures.

(5) Bilaterally adrenalectomized "depleted" rats failed to show a rise of urinary nitrogen on reduction of caloric intake. There was no rise in N.P.N. in these animals. This, together with conclusion 7, strengthens the belief in the existence of a role of the adrenal cortex in this metabolic response after a damaging stimulus, including that of starvation.

(6) It was suggested that possibly the economy of the "depleted" rat's metabolism accounted for the lack of increased output after a damaging stimulus, whereas in starvation the cortex now responded with consequent catabolism of its body tissue. It was further shown that the adrenal of two previously "depleted" animals underwent comparative hypertrophy after showing a "pre-mortal" rise in urinary nitrogen.

(7) Whereas unilaterally adrenalectomized adult rats, fed an adequate diet, showed the characteristic rise in urinary nitrogen after the operation, completely adrenalectomized adult rats fail to present this postoperative metabolic picture, in fact a considerable fall in the level of nitrogen excretion occurred, and further a fracture of the left femur of totally adrenalectomized rat fails to evince any rise in nitrogen excretion even in the face of an inadequate (maintenance) protein and caloric intake.

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