THE EFFECT OF ADRENOCORTICOTROPHIN ON PLASMA PROTEIN REGENERATION IN THE RAT FOLLOWING DEPLETION BY MASSIVE HEMORRHAGE

by

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THESIS

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

There have been many attempts to elucidate the dynamics of the repeatedly observed increased urinary nitrogen exertion following the administration of adrenocorticotrophin and adrenal steroids.

Dr. J. S. L. Browne postulated that these hormones might exert their effect by mobilizing, or "loosening", the body tissue proteins from the internal body sources for the purpose of supplying protein building materials to loci where they might be needed (1).

It was suggested that this hypothesis might be tested in the plasma protein depleted rat by studying the effects of these hormones on the regeneration of plasma protein. The stimulus of hypoproteinemia in such an experiment would seem to lend itself especially well to the study of these hormones on protein metabolism. The plasma protein, according to the above hypothesis, would become the anabolic focus relative to the tissue proteins, and any resultant anticipated effects would be in accordance with Whipple's theory of a dynamic equilibrium between tissue and plasma proteins (2, 3).

HISTORICAL REVIEW

A review of the voluminous literature over the past fifty years, pertinent to protein metabolism, attests to the efforts of investigators to elucidate this complex subject. While much has been learnt, there remains innumerable problems yet to be resolved before a satisfactory understanding will be realized.

Perhaps one of the most important recent approaches to this problem has been the studies on the relationship of the endocrines to protein metabolism, particularly the adrenocortical hormones. Although the work in this paper deals primarily with their effect on plasma protein regeneration, it is apparent that any understanding of the subject of plasma protein formation warrants a consideration of the general topic of protein metabolism. An extensive historical review is obviously beyond the scope of such a paper, and only the major contributions leading to our present knowledge will be considered.

SITE OF FORMATION OF PLASMA PROTEINS

The literature is abundant with conflicting reports regarding the site of formation of plasma proteins. However, many persistent workers, such as Dr. G. H. Whipple, feel that the liver is the master organ in the production of plasma proteins.

Fibrinogen

Studies on fibrinogen indicate that this protein is formed entirely in the liver. In 1905, Doyon showed that by producing extensive degenerative changes in the liver with chloroform and phosphorus there was a fall in blood fibrinogen, and suggested the liver as the site of its formation (4, 5). In attempting to find out why operated animals bled following chloroform poisoning, Whipple and Hurwitz (6) found that the level of blood fibrinogen paralleled the extent of liver damage, with a maximum decline observed in two days. Furthermore, this relationship existed with healing of the liver. Others have corroborated these observations following liver poisons (7, 8, 9). Goodposture (10) has shown that after defibrination of the blood there was a marked decline in fibrinogen regeneration, in contrast to the rapid regeneration which occurred in normal animals (11, 12, 13). Drury and McMaster observed that after 90% removal, there was complete regeneration of fibrinogen in 5 to 6 hours (11).

Conclusions drawn from the use of liver poisons have been questioned on the grounds that these poisons were not localized in their actions to the liver (14). However, it has been shown that direct injection of the poison into the portal vein, causing infarct like lesions in the liver, produces a decline in the blood fibrinogen (15).

Studies on Eck fistula dogs and various other modifications of this procedure, such as hepatic artery ligation and exclusion of the intestine from the circulation, have yielded conflicting results (16, 10, 17, 18). Goodposture (10) and Meek (18) concluded from their results that fibrinogen was dependent on the combined activity of liver and intestine.

The results following total hepatectomy support the concept that fibrinogen is formed in the liver (12, 13, 19, 9, 11). Drury and McMaster showed after hepatectomy in rabbits that there was a fall in blood fibrinogen of 35 to 65% below normal in 15 to 30 hours, and that after 90% defibrination the level remained relatively low, in contrast to the rapid regeneration in normals (11). Similar results were found in hepatectomized dogs (19). Further recent evidence, based on observations of perfusion experiments of the isolated intact liver with isotopic labelled amino acids, has indicated that the liver synthesized all the plasma fibrinogen (20).

Almost every organ in the body has been considered on the basis of minor changes in fibrinogen levels. This accounts for the conflicting reports in the literature. The marked lability of the plasma fibrinogen in dogs has been frequently observed (21, 22), with a normal variation of 20 to 25% shown by Foster and Whipple (22). Under such experimental conditions as hemorrhage, blood transfusions (8, 24) and tissue injury (16, 15, 8), there are marked variations in the plasma fibrinogen. Elevated levels have been found after small doses of liver poisons (8), and immediately after hepatectomy (13). The difficulty in accurate measurement (23) and the rapidity with which fibrinogen regenerates (11, 13, 24, 19) also explain the conflicting reports in the literature. When cognizance of these facts is taken, the discrepant reports in the earlier literature (17, 16) can be more completely evaluated.

Albumin and Globulin

There is still less agreement regarding the site of origin of albumin and globulin. Workers such as Whipple (25) and Elman (26) feel that the liver is the site of formation of all the albumin and most of the globulins; while others have suggested that the evidence is insufficient and feel that other tissues, such as muscle and connective tissue, should be studies (27, 28).

Kerr, Hurwitz and Whipple showed that phosphorus and chloroform poisoning in dogs were associated with a fall in plasma protein, and that regeneration was delayed after depletion in those animals in which severe liver damage was found (29). They also noted that Eck fistula dogs were unable to regenerate serum proteins after acute plasma depletion experiments, and concluded that the liver was concerned in maintaining the normal level of plasma protein. These findings were later confirmed by Whipple (21, 25) who also showed that when anemia was produced in Eck fistula dogs the capacity to form new hemoglobin was only one fourth that of the normal (25). He suggested that the liver was directly or indirectly concerned with the production of new hemoglobin.

The evidence from hepatectomy experiments is conflicting. In partially hepatectomized rats a fall in the total proteins and a rise in the globulins were noted (30, 31). It was also observed that the plasma protein returned to normal concomitantly with restoration of the liver to normal weight (31). Berryman, Bollman and Mann were unable to verify these findings in short term experiments, but after plasmapheresis they noted absence of regeneration up to nine hours after depletion (32). Observations of other workers led them to minimize the role of the liver in plasma protein formation and to suggest that protein enters the circulation from other sites (33, 34). Munro and Avery were

unable to find any marked changes in the relative concentrations of the various components of plasma by electrophoresis in hepatectomized dogs (34).

Recent studies have offered further support for the liver as the site of plasma protein formation. Tarver and Reinhardt (35) fed methionine labelled with radioactive sulfur to hepatectomized dogs and found that while it was incorporated into the body proteins to the same degree as in normals, the rates of albumin and globulin synthesis were respectively 20 and 7 times greater in the normal dogs. This indicated that globulin can be formed in the absence of the liver. Intravenous injections of acacia, which is stored in the liver, were found to produce hypoproteinemia (36, 37). This was felt to be due to interference with production, or release, of protein from the liver.

Information obtained from the studies of liver slices has yielded additional support (38, 39, 40). Peters and Anfinsin incubated liver slices with radioactive labelled amino acids, and found by fractionation that the protein entering the media yielded a fraction 8 to 16 times the specific activity of the slices. Immunological, ultracentrifugal and electrophoretic examination showed that this fraction consisted mainly of serum albumin (40). Experiments by Miller, who perfused the isolated intact liver with labelled amino acids, indicated that the liver synthesizes practically all the plasma fibrinogen, the albumin and probably 80% of the plasma globulin (20).

There is much clinical evidence from the study of liver disease to support the liver as being the site of origin of the plasma proteins (41, 42, 43, 44), since nitrogen balance studies in such patients show that they are able to store nitrogen, but have difficulty in synthesizing plasma protein. Two cases of idiopathic hypoproteinemia were studied which showed liver atrophy (45) and interstitial hepatitis (46) at autopsy. Later, Rytand (47) reported a case of idiopathic hypoproteinemia, but was unable to find any liver or other disease to account for it. He suggested that this may be an exaggeration of the varying abilities of different individuals to form protein. The inadequacy of the "loss and lack" theory to explain hypoproteinemic states has been frequently pointed out (48, 49).

There is little doubt that the reticulo-endothelial system forms globulins, and this view has been accepted by most investigators today. The clinical observations of hyperglobulinemia in diseases which involve the reticulo-endothelial system (33, 42), and the lack of correlation between albumin and globulin levels in malnutrition states (41), draw attention to other sites of formation, such as the R.E. system.

The relationship between antibody formation and the globulin fraction of the plasma has been well established. Avery (50) demonstrated this by studying antipneumococcal serum; and subsequently it was shown that the antibody was a protein (51, 52, 53). This has been confirmed by investigation of the physicochemi-

cal proterties of antibodies by the ultracentrufuge (54) and by electrophoresis (55), whereby they have been shown to be globulins. Heidelberger (56), using quantitative precipitin tests, has shown that antibodies may constitute 60 to 70% of all serum globulin in highly immunized animals.

Many investigators have tried to define clearly the site of antibody formation. Early workers suggested they were formed from disintegrated leucocytes (57), while evidence from more recent studies has shown that the reticulo-endothelial system, particularly the lymph and lymphocytes, are concerned in the formation of gamma globulin (58, 59, 56, 60, 61). Sabin has obtained visual evidence by administering a conjugated protein antigen with a visible dye. Corresponding with the time of antibody formation, the cells which took up the dye appeared to shed their surface film into the surrounding fluid, rendering the dye invisible (58). She accordingly concluded that since the R.E. cells formed the antibody and the antibody resides in the globulin fraction of the blood proteins, these cells might be the origin of normal serum globulin.

Other workers, in recent studies, have renewed the theory that the function of antibody and globulin formation resides in the leucocyte (62, 60, 63, 61) and in the granulocytes and macrophages (59). Studies of pathological states in which there is a hyperglobulinemia and plasma cell proliferation, as

in multiple myeloma (64, 65, 66) and in immune states (64), have led to the belief that the plasma cells form the globulin fraction.

There does not seem to be much disagreement with the fact that globulins can be formed by the R.E. system, although some authors have attempted to designate it as an important site of all plasma protein production. Cutting & Cutter (67) injected india ink into rats and found that it inhibited the normal rapid regeneration of plasma protein after hemorrhage. Landsteiner & Parker have attempted to show that explanted connective tissue cells are capable of synthesizing and breaking down blood proteins (68). Recently Cheng, in a critical review of the subject, attempted to repeat all the earlier work with liver poisons, Eck fistula and hepatectomized dogs (27). His failure to corroborate previous observations led him to minimize the role of the liver in plasma protein formation. Nevertheless, there is abundant evidence to suggest strongly that the liver is probably the most important site of formation of all the plasma proteins, with the R.E. system contributing a part of the globulin fraction.

THE EFFECT OF FOOD PROTEIN ON PLASMA PROTEIN REGENERATION

It is well known from clinical (69) and experimental (70, 71, 72) studies that diet greatly influences the production of plasma protein, and that food protein has a qualitative as well as a quantitative importance.

In 1918, Kerr, Hurwitz and Whipple (73) showed in plasma depleted dogs that regeneration was more rapid when animals were fed than when fasted, and that regeneration was faster on a meat than a milk diet. The rapidity with which dietary proteins correct serum albumin depletion has been more recently demonstrated by Elman (74).

Many workers, using different techniques, have studied the potency of food proteins by their ability to regenerate plasma protein. Whipple and his co-workers (70) used the technique of plasmapheresis, by which the plasma proteins are removed by daily bleeding and the red cells reinjected. The plasma protein level is reduced to 4 grams per 100 ml. and maintained at that level until a constant minimum quantity of plasma removal is required. This amount is considered to be the basal output. Various foods were added to the basal diet and the amount of plasma protein, over and above the basal output, required to be removed to maintain the constant level, was used as an index of the potency of the food protein. This was expressed as the weight of new plasma protein resulting from the feeding of 100 grams of the

test protein. It was found that beef serum and grain products were the most potent and kidney the least, with the other proteins such as liver, casein, beef heart and skeletal muscle falling in an intermediate group.

Weech and Goettsch obtained similar results, but with a wider variation in the different animals tested (75, 76, 77). They fed dogs an inadequate protein diet for three weeks, then added the test protein for one week and measured the effect on serum albumin concentration. In contrast to Whipple's technique, they measured rate of formation and not total capacity for protein formation. Melnick, Cowgill and Burack (78), using a different plasmapheresis technique, were unable to find any difference in potency of different food proteins, probably due to the inaccuracies of their method (2). More recent experimental work, however, by Cannon (79), Chow (80) and Whipple (81) has confirmed the earlier findings of Whipple and Weech. Cannon (82) has shown the impaired ability of hypoproteinemic rabbits to produce agglutinins while on low protein diets.

These studies have led to the investigation of whether certain food proteins favour the production of particular blood proteins. Liu, in 1932 (83), found animal protein twice as effective as vegetable protein in combating edema and hypoproteinemia, although subsequent work from his laboratory (84) showed only a slightly more favourable action of animal protein in raising the

plasma protein concentration of two patients with nephrosis.

Whipple studied the problem and in his early work (85) suggested that certain diets such as vegetable protein favoured globulin formation, while animal proteins such as liver and casein favoured albumin formation. During depletion by plasmapheresis (2) and dietary restriction (86, 87) there is always a lowered albumin-globulin ratio, regardless of diet; but it was greater when grain or plant proteins were fed (70, 71), except for soy bean which is in a class by itself and favours a high A-G ratio (88).

In the studies of Weech and co-workers (75), the interpretation of their results led them to believe that the globulin fraction of the plasma was not effected by diet, postulating that the internal mechanism controlling the formation of albumin and globulin was different. However, the work of Whipple's group clearly shows that diet alone does affect globulin formation (85, 70). They found that the feeding of 100 grams of beef serum produced 21 grams of albumin and 17 grams of globulin (70), while the feeding of a similar amount of bran flakes, added to a basal kidney diet (88), resulted in the formation of 12 grams albumin and 11 grams globulin.

The limitation of these studies were the inherent inadequacy of the chemical methods for the determination of albumin and globulin content, and the lack of information regarding plasma volume. With the availability of electrophoresis and improved

methods for determining the plasma volume, Chow and his co-workers reinvestigated the nutritive properties of proteins with respect to their ability to support nitrogen balance, to promote growth, to replace body tissue, to regenerate liver proteins and to stimulate the production of plasma proteins (80). These properties paralleled each other in all the individual proteins tested, with the exception of egg-white, and all the proteins were noted to vary in their potency. It was further shown by these workers that lactalbumin, or its hydrolysate, favoured the regeneration of albumin, whereas the others tested regenerated globulin equally well. Examination of the A-G ratio after repletion demonstrated that the ratio returned to normal after the feeding of lactalbumin, its hydrolysate and casein hydrolysate.

AMINO ACID REQUIREMENTS IN PLASMA PROTEIN REGENERATION

The recognition that different protein foods varied in their potency in providing the nitrogen requirements of the body, has led to investigation of the amino acids. Accordingly, amino acid mixtures and protein hydrolysates have been assayed by different biological methods.

Rose studied the amino acid requirements for the normal growth of rats and found that a mixture of the following 10 amino acids, in appropriate quantities, were indispensable for normal growth: threonine, valine, leucine, isoleucine, methionine, phenylalanine, tryptophane, arginine, histidine and lysine (89, 90).

The availability of protein hydrolysates as a source of amino acids has led to extensive investigation of their use in the regeneration of plasma proteins. It has been quite clearly shown by many investigators that these materials are capable of producing regeneration of plasma protein in depleted dogs (91, 92, 93, 94, 95, 96) and in depleted rats (97), whether administered parenterally or orally. There is also clinical evidence to show that these extracts are well utilized, as indicated by nitrogen balance, regeneration of serum proteins and by reduction of nutritional edema (98, 99, 100). Elman (91) showed, in dogs made hypoproteinemic after a single hemorrhage, that

hydrolyzed casein given intravenously produced a 2.6 to 2.95 grams per 100 ml. increase in the albumin fraction. In studies of patients suffering from malnutrition and hypoproteinemia, infusions of casein hydrolysate caused edema to disappear, the serum protein to rise and a positive nitrogen balance, but only if tryptophane and cystine (or methionine) were added to the digest (98). The studies of the 10 essential amino acids necessary for growth in rats were extended to other animals. Elman (101) showed that following a single hemorrhage in normal dogs, intravenous injections of the amino acid mixture caused a marked increase in plasma protein regeneration. Later, Rose (102) showed that the mixture of the essential amino acids was also indispensable to dogs, as measured by nitrogen balance studies and weight gain, and he accordingly suggested that the qualitative amino acid requirements of all mammals might be the same.

Using the plasmapheresis technique, Whipple and his coworkers have made extensive studies of Rose's essential amino acids on the regeneration of plasma proteins (103, 104, 105, 106). It was shown that cystine and tyrosine, or tryptophane, adds much plasma protein producing power to gelatin and zein (up to 25 to 40%), and that methionine could not substitute for cystine, nor phenylalanine for tyrosine (103). In later experiments, different amino acids were omitted from the mixture and an impairment in plasma protein formation was noted, as well as changes in nitrogen

balances and weight (105, 76). They found that omission of leucine or isoleucine resulted in negative nitrogen balance and weight loss, but that plasma protein regeneration was maintained (105). They thought that leucine may be obtained from the disintegration of red blood cells or tissues, since after restoring the deficient amino acids there was a slight delay in the expected plasma regeneration, presumably due to the priority of tissue repair. Studies in rats show a similar impairment of plasma protein regeneration following the omission of any of the essential amino acids from the mixture (107, 108). These mixtures have been administered with good results orally and parenterally, although their action has been found (105) to be more effective orally.

Clinically, the amino acid mixture has been found to promote plasma protein regeneration (109) and to maintain nitrogen equilibrium (110). It has been suggested that the failures of amino acid therapy in hypoproteinemia are due to the inability of administering enough calories parenterally to prevent deamination of the amino acids (111). To accept this as an explanation for the poor results of amino acid therapy in hypoproteinemia would belie the complexity of the problem.

That there are other unknown nutritional factors in protein synthesis has been suggested by the studies of Woolley (112, 113), and Womack and Rose (114). Mice were found to grow

at a suboptimal rate on the amino acid mixtures, as well as on casein hydrolysate fortified with cystine and tryptophane; but when small amounts of intact casein or crystalline trypsinogen were added there was a restoration of normal growth (112). The unknown substance, called strepogenin, was prepared from a tryptic digest of casein, and when added to the diet it caused normal growth. It was furthermore noted that strepogenin was found in casein, but only in traces in egg-white, and that its activity was lost by acid hydrolysis. Other workers have suggested the possible existence of an unknown growth factor on the basis of impaired plasma protein regeneration (105) and weight gain (118), observed during a dietary deficiency not related to the proteins or amino acids.

ENDOGENOUS MATERIALS CONCERNED IN PLASMA PROTEIN FORMATION

There is ample experimental evidence to show that there are dispensable and indispensable stores of proteins in the body (3). As early as 1906, Morowitz showed that regeneration of blood plasma proteins occurred in the fasting animal after acute depletion by bleeding, as evidenced by a rise from 2 grams per 100 ml. to normal levels in 2 to 4 days (124). Whipple's studies on plasma protein depleted and fasting dogs offered further support to indicate that plasma protein regeneration can occur in the fasting animal (115, 116). Similarly, Cutting and Cutter found in rats that 40% of the original mass of circulating protein could be regenerated in 12 hours after its partial removal, even if the animals were fasted for 7 days previously (67).

Whipple and his co-workers attempted to measure the reserve stores of protein in the dog available for plasma protein formation (85, 71, 88, 70, 117). It was noted that following depletion of circulating plasma protein by daily plasmapheresis, while on a basal low protein diet, it was necessary to remove larger quantities of plasma protein in the initial weeks of the experiment than in the later periods, in order to obtain and maintain a constant low level of hypoproteinemia (117). This

excess quantity removed in the initial period was considered to represent the reserve store, and to arise from protein materials present in the body at the start of the experiment. The maximum quantity of these stores was not measured, since the animals were usually fasted during the initial period or depleted previously by a low protein diet. Thus, Whipple felt that the measurements were lower than the true values. It was estimated that normal dogs have storage materials sufficient to form an amount of plasma protein one to two times that normally present in their circulation. This amount was in excess of the total protein content of the liver, and it was suggested that a large portion must be stored elsewhere (2). Furthermore, the amount so estimated was larger than the amount of plasma protein regenerated on a fasting regime (115, 116), and probably more accurately represented the amount which could be produced when plasma regeneration was the only demand on the protein stores.

Whipple studied the process of storage (117) by discontinuing the plasmapheresis in the depleted dog. The amount of plasma protein which would have had to be removed to maintain the steady hypoproteinemic level was stored in the body, and equalled 150% of the entire mass of circulating protein before storage.

More recently, Whipple's laboratory has attempted to study the maximal hemoglobin and plasma protein production in dogs under the stimulus of double depletion (119, 95, 120). They have shown that the ceiling for hemoglobin formation in anemic dogs is one gram per kilo per day on high protein diets and intravenous iron, and 1.5 grams per kilo per day in doubly depleted dogs (119). The actual maximal plasma protein production in hypoproteinemia was believed to be about one gram per kilo per day, but the true ceiling could not be determined by this technique. It was impossible to remove the new plasma protein as fast as it was formed, and the hypoproteinemia could not be maintained constant in the face of a rich dietary intake. It was furthermore demonstrated that the circulating protein pool was contributing to tissue protein, as indicated by the positive nitrogen balance and weight gain.

Further evidence to support the concept of a labile protein store was demonstrated in man by the simple method of administering normal saline intravenously and measuring the plasma volume and plasma protein (121). In normals there was a rise in the plasma volume of 441 ml. and an increase in total circulating protein of 13.9 grams, in contrast to the undernourished patients who showed a rise in the volume of 170 ml. and a fall in the total circulating protein of 14.7 grams.

An important recent development in amino acid nutrition is the demonstration that non-essential amino acids can be synthesized from glutamic acid and the unnatural forms of the essential

amino acids (125), from ammonium citrate (126) and from glycine and urea (127). The utilization of ammonia, but not urea, for amino acid synthesis has been confirmed by the use of appropriate dietary supplements labelled with N 15 (128, 129, 130). The implication of these studies relative to plasma protein regeneration are quite apparent, although not completely worked out.

There is strong evidence for the presence of a body source of materials available for plasma protein formation. It would be difficult to suppose that these stores are any different from the general body stores which have been measured in the rat by Addis (122, 123).

NATURE OF THE PROTEIN RESERVE STORE

Despite the available evidence that there are reserve endogenous materials available for the formation of proteins, the nature of these "reserve stores" has not been fully determined.

The location of the protein stores in the body is quite general, but the outstanding role of the liver seems clear (122, 131, 132, 123, 133). Addis, Poo and Lew (122) measured the loss of protein in a rat during a seven day fast and found that muscle, skin and skeleton contributed 62% of the total, liver 16% and alimentary tract, pancreas and spleen 14%. The proportion of original protein content lost by various organs were widely different, but the highest was the liver with a 40% loss.

These workers further noted that, during a seven day fast, half of the total loss of protein occurred in the first two days (131), indicating the lability and rapid availability of these stores. This early rapid rate of plasma protein regeneration from the body stores has been frequently observed in the plasmapheresed dogs (124, 116, 117) in which the rate of regeneration was most rapid during the first few hours (124, 116). In attempting to deplete the reserve stores, it was noted that the greater amount was removed during the first week (117).

The manner in which protein is stored in the body is not understood, although many interesting hypotheses have been presented. Borsook and Keighley (134), in a review of their own data and of others, concluded that heterogeneous metabolites are stored as protein. Other workers were unable to find evidence for such a preformed store of plasma protein (135, 2). Whipple and Madden have suggested that the rate of restoration of plasma protein, following acute depletion in dogs, indicates that a greater bulk of the reserve stores does not exist as preformed plasma protein (2). These workers believed that if a large protein store did exist in the preformed or modified state, an even more prompt return to normal of the plasma protein concentration could be anticipated. This was not found, since only a small rise of 0.5 grams per 100 ml. was noted within fifteen minutes after rapid depletion (116). However, dietary proteins (73) or intravenous amino acids (136) did accelerate the relatively slow regeneration of plasma protein from reserve stores.

Attempts to distinguish by chemical or physical means a tissue protein, or any protein, which might have the distinctive position of being reserved or stored protein have not achieved any success. Luck (132, 137), in his chemical studies of liver protein after storage, provided evidence to indicate that all protein fractions took part in the storage process, and that no

single protein or fraction of the hepatic proteins could be regarded chemically as a distinctive storage protein different from the basic structural protein of the organs. Recent electrophoretic studies in plasmapheresed dogs during depletion and repletion have not offered any further information regarding the nature of the protein stores (138, 139, 140).

The evidence from Whipple's laboratory has suggested the existence of so called "dispensable and indispensable" stores of protein in the animal body (3), in the course of their work on hemoglobin and plasma protein regeneration. These dispensable stores are readily raided when emergency demands for specific amino acids, or for proteins, are not covered by the food supply (70, 85, 71, 88). The indispensable or essential body protein is believed not to partake in the formation of plasma proteins. The small amount of protein (3 to 4 grams) required to be removed from the protein depleted dog to maintain a constant hypoproteinemia, during a week of fasting, appears to be in accord with this (117, 103 , 88, 70). This amount was considered attributable to a carry over from providing basal diet feeding, to changes in plasma volume and to other uncontrollable variations in basal conditions. Whipple and his co-workers have accordingly defined the reserve or indispensable store of protein as, "all proteins which may be given up by an organ or tissue, under uniform conditions, without interfering with organ or body function" (2). In certain emergencies, however, such as

in the doubly depleted dog with anemia and hypoproteinemia and on a fasting diet, the dog is able to produce new hemoglobin and plasma protein (120). The authors have attempted to explain this raiding of the body stores on the basis of the priority of circulating blood proteins over the tissue and organ stores, in the presence of an acute emergency.

OTHER FACTORS AFFECTING PLASMA PROTEIN FORMATION

It is well known that tissue injury markedly affects the plasma protein forming mechanism. In rats it has been shown that procedures such as laparotomy caused reduction of plasma albumin and an increase in the globulin fraction (30). Madden and Whipple, in studying these factors in plasmapheresed dogs consuming an ample diet, showed that sterile turpentine abscesses and induced digestive disturbances caused marked reduction in plasma protein regeneration (88, 71, 117). The lability of serum fibrinogen in infections, trauma and intoxication has been repeatedly demonstrated (16, 8, 15).

The alteration of the albumin-globulin ratio with intoxication and infection has been demonstrated to occur as a result of an increase in the globulin fraction (141, 57). The plasma of dogs which were subjected to various injuries and to toxins, such as the nitrogen mustards, has been analysed electrophoretically. A fall in the albumin and an increase, particularly, in the alpha globulin concentration have been shown (142, 143).

Clinical studies following injuries have demonstrated similar alterations in the plasma proteins (144). Reviews of the clinical significance of serum proteins (145) and nitrogen metabolism in disease (159) reflect the inadequate understanding

of the dynamics of these alterations. The internal mechanism of body protein formation can be disturbed by a variety of factors, but the exact nature of their action is still not clear.

MECHANISM OF PLASMA PROTEIN FORMATION

There is little information regarding the exact mechanism of plasma protein formation. It is generally assumed that the amino acids liberated by protein digestions are the ultimate sources of the essential nitrogen (146, 3). It is also believed that the amino acids are the simplest units of protein synthesis, although this assumption has been questioned (147). The evidence from Whipple's studies suggests that ingested food is absorbed from the gastro-intestinal tract in the form of amino acids, and that the liver, which is the master organ in protein synthesis, aggregates the amino acids and other nitrogenous substances into proteins (3). As early as 1913, Van Slyke (148) offered evidence through tissue examination to suggest that the absorbed amino acids were not stored in the tissues in this form, but as proteins. Whether the materials available for plasma protein formation is stored as such, or as protein aggregates which undergo modification, is not known.

The difficulties in working out the problem of the mechanism of plasma protein formation are quite apparent, when the dynamic state of body protein is recognized.

CONCEPT OF THE DYNAMIC STATE OF BODY PROTEINS

The relationship of the plasma proteins to general body protein metabolism requires consideration in order to facilitate an understanding of the dynamics of plasma protein formation. The investigations of Schoenheimer (149), Borsook (150) and Whipple (3) have revolutionized the early conception of the exogenous metabolism of Folin (151). They have revealed a dynamic, rather than a static state of tissue proteins. The role of plasma proteins as "part of a balanced system of body proteins" in which there is a constant "ebb and flow" between it and the body stores has been postulated by Whipple (152, 3).

Folin's original concept, that there were two kinds of metabolism which were essentially different and independent, was based on the observed differences in the urinary constituents when the total nitrogen output was varied by diet. One type which yielded urea and inorganic sulphates was considered variable in quantity; and the other, yielding creatinine and neutral sulphates, constant. He named the former type of metabolism, exogenous or intermediate metabolism and the latter, tissue or endogenous metabolism (151). The essential constancy of the endogenous protein catabolism (153), as well as the independence of the endogenous and exogenous metabolism of nitrogen (154), has been repeat-

edly confirmed. Later developments, however, have shown that the rate of turnover of body proteins in animals in nitrogen balance is far greater than was envisaged by Folin's theory.

The evidence upon which the concept of a state of dynamic equilibrium of body proteins exists, has arisen from experiments which have upset the dynamic equilibrium. In the previous studies it was clearly demonstrated that the body protein may flow from the tissues or cells to the plasma, and that the supply of body proteins ultimately depends upon the exogenous sources. Further experiments have shown that proteins may flow in the opposite direction, that is, from the circulating pool to the tissues.

It has been demonstrated that the intravenous administration of dog plasma, as the sole source of nitrogen in protein depleted dogs, is well utilized to maintain nitrogen equilibrium from 14 to 20 days (152, 155, 156, 305). Addis (157) found an increase in the protein content of tissues following intraperitoneal injection of plasma in rats. Howland and Hawkins (158) noted that plasma administered intravenously to phlorhizinized dogs did not cause an increase in blood amino acids sufficiently large to be detected as increased nitrogen and dextrose. However, when it was given orally a significant rise was found. These observations have led to the suggestion that there is a partial catabolism of the injected proteins to large aggregates, with reassembly by the

cells to form their own particular type of protein, without complete catabolism to amino acids and the subsequent resynthesis to protein (70, 158, 3). That this participation in body metabolism is accomplished without evidence of profound protein breakdown, is attested to by the relative conservation of nitrogen which occurred during and after parenteral plasma administration, as well as by the marked difference in the behavour of plasma protein given orally and intravenously.

Recently, Whipple and co-workers have given plasma labelled with isotopic carbon intravenously to dogs. They have found that its incorporation into tissue proteins was attended by only very small losses of activity in the urine (163), again providing no evidence for complete breakdown to amino acids. The manner in which these protein aggregates traverse the cell boundary is still conjectural (3).

Clinical studies by nitrogen balance methods have not offered any further information regarding the fate of intravenous plasma (160, 161, 162). Eckhardt, however, claims that a slow degradation of plasma protein, together with a gradual release and more or less complete conservation of amino acids, precedes the utilization by organ and tissue cells (161).

The investigations of Schoenheimer and his group on intermediary protein metabolism, using amino acids labelled with N 15, have further revealed the dynamic rather than the static

state of tissue proteins (149). They have shown that the nitrogenous groupings of tissue proteins are constantly involved in chemical reactions, whereby peptide linkages open and liberate amino acids which mix with others of whatever source, dietary or tissue. These amino acid molecule mixtures partake in a variety of chemical reactions, such as entering open peptide linkages, or transferring nitrogen to deaminated molecules to form new amino acids. The cycle continues, thus concealing the original source of the nitrogen.

In the light of the experimental evidence Whipple's theory of the body proteins being in a state of dynamic equilibrium (3) appear tenable. Nevertheless, it leaves many difficult unsolved problems in cellular metabolism to be explored.

THE RELATIONSHIP OF PLASMA VOLUME TO PLASMA PROTEIN CONCENTRATION

In the studies of protein metabolism, the measurement of plasma proteins has usually been expressed in terms of concentration of the plasma protein rather than the absolute quantity. It is apparent that changes in the plasma volume might significantly mask an absolute change in the plasma protein which would not be reflected in the relative measurements of concentration (181, 182, 164). In view of the nature of the following experiments, and the fact that specific variations in the total amounts of circulating protein will usually become evident before the alteration in concentration (183, 181, 184), the relationship of plasma volume to plasma concentration and total circulating protein warrants consideration.

Variations of plasma volume have been found under a variety of conditions, such as changes in climate (166), drug administration (165, 167), anaesthesia (168), growth (175, 176), changes in posture (177), nutritional states (169, 178, 179, 180), surgical procedures (174, 169) and pregnancy (173). With such variations in plasma volume consideration must be given to the possible concomitant changes in the total circulating protein, in attempting to assess the circulating protein pool. That alterations in plasma volume may mask the changes in total circulating

proteins, which are not detectable by measurement of concentration, have been demonstrated by the administration of drugs which alter plasma volume (167), following hemorrhage (172, 171) and transfusions (170), by changing the temperature of the environment (166) and after surgery, dehydration and malnutrition (169).

It has been shown by the administration (185, 186, 187) and removal (188) of protein that the plasma volume increases and decreases as the total amount of circulating proteins is increased or decreased, even when the volume withdrawn is replaced by fluid. Following hemorrhage (172, 189) the gradual rise in plasma volume due to hemodilution was accompanied by an increase in the plasma protein concentration, particularly the albumin fraction (189). This was considerably less than would be expected from the changes in the plasma volume. These changes were not permanent, since the homeostatic mechanisms of the body were able to add or remove proteins from the circulation. That there are other mechanisms has been suggested by Stead. He has indicated that a marked decrease in the osmotic pressure of the plasma can be compensated for by a considerable increase in the pressure of the extracellular fluid, without affecting the circulation or size of the plasma volume (169).

In studies of induced acute and chronic dietary hypoproteinemia in rats, by Metcoff, Favour and Stare (164) and

others (179, 193), a definite contraction of plasma volume and total circulating proteins per unit of body surface was found. There were minimal changes only in the plasma protein concentration in the acute experiments, and no change in the chronic ones. Similar results have been obtained in hypoproteinemic dogs (194, 195). That changes in the concentration of protein are not a good index of changes in plasma volume has been further shown by observations in humans (190, 166, 191). An increase in plasma volume of 29.5% was accompanied by an increase of plasma protein concentration of only 3.4%, and similarly a decrease in volume of 31% was associated with a rise in concentration of only 5.4% (166). Others have also noted alterations in plasma volume far greater than would be expected from the slight changes in the serum protein concentration (191, 190). The results of these observations in humans have led to the conclusion that serum protein concentration is not altered in proportion to the changes in plasma volume, due to the fact that protein is added to the plasma with increases in volume, and removed from the plasma with decreases in volume (190). This would be also in accord with Whipple's theory of a dynamic equilibrium (2).

It appears from the above evidence that consideration of concentration alone is unphysiologic, for, as Peters points out, "a three dimensional function can not be evaluated by two dimensional measurements, and alterations in total circulation protein

and plasma volume can not be evaluated from total protein concentration" (192).

THE INFLUENCE OF HORMONES ON NITROGEN METABOLISM

The hormones function as homeostatic regulators of physiologic reactions and would be expected to exert as influence on protein metabolism which, according to the work of Whipple (3) and Schoenheimer (149), is a dynamic process. The fact that hormones affect processes which may continue even in their absence, and that their actions may be directly on protein metabolism or indirectly through some metabolic process other than protein metabolism, complicates the understanding of their role (200). Moreover, the inter-relationship of the hormonal system adds greatly to the problem of understanding their actions.

It is beyond the scope of this paper to survey all the well known clinical and experimental evidence demonstrating the influence of hormones on protein metabolism. Accordingly, only a review of such facts which may be relevant to the dynamics of plasma proteins will be considered briefly, with attention devoted mainly to the adrenocorticotrophins and adrenal steroids.

Recent studies by Szego and White (196) have shown that the primary action of growth hormones is on the liver, and that it is manifested by a mobilization of lipid. It was suggested that the protein sparing action of the lipid made available a greater proportion of amino acid nitrogen for the synthesis of new proteins, as well as providing energy yielding systems

necessary for protein synthesis. In view of the earlier studies (197) which demonstrated that growth is accompanied by an increased deposition of protein and water, together with a diminished deposition of fat, this concept seems quite plausible. These data would suggest that the action of growth hormones is in some way associated with protein metabolism.

The well known effect of the thyroid gland on oxygen consumption would naturally direct attention to its effect on the rates of protein metabolic processes. The anabolic effect of thyroid has been demonstrated by inducing positive nitrogen balance in growing children following the administration of small amounts of thyroid (198). That there is a catabolic effect has also been shown by the aggravation of diabetes in hyperthyroidism (199). This would suggest an increased transformation of protein into carbohydrates. The mobilization of protein with degradation to amino acids has been suggested as the possible role of the thryoid in protein metabolism. This has been based on the observation that fasting thyroidectomized animals excreted less sugar and nitrogen under the influence of phlorhizin than did the phlorhizinized controls; but when protein was fed, there was no difference in excretion (201, 202).

Kochakian and Murlin (203, 204) in their experimental studies have shown the marked effect of androgens on nitrogen retention. This was unaccompanied by any rise in the nitrogenous substances of the blood. That this nitrogen retention was not due

only to growth of accessory sex organs was also shown (205). This subject has been extensively reviewed by Kenyon (217, 206, 207, 208) and the observations confirmed in a variety of laboratory and clinical studies. Abels (209) has shown that testosterone injections caused nitrogen retention in normal and abnormal subjects, and that there are initial decreases in the total amount of circulating protein. This suggests tissue protein synthesis or inhibition of plasma protein formation.

The local effect of estrogens in causing proliferation of certain specific tissues such as the uterus, mammary glands and bone is well known, and in a sense represents an anabolic function (210). With respect to the definite anabolic effect on bone metabolism, Albright (211, 212) has suggested that the primary action of estrogens in osteoporotic women is an anabolic one on bone matrix, with a secondary retention of calcium and phosphorus. Kenyon (206) has shown that estrogens administered to sexually underdeveloped women and to normal women exhibit metabolic effects similar to testosterone, in that there is retention of nitrogen, inorganic phosphorus and sodium. However, a catabolic effect of estrogens in protein metabolism has been demonstrated in rats (218) and in partially depancreatized ferrets (214). It is not known whether this is a primary effect, or a secondary one mediated through the adrenal cortex.

There is evidence to show that insulin administration to the diabetic and normal animal, in the presence of an ample

carbohydrate diet, decreases the urinary nitrogen excretion, and in large doses increases the excretion of nitrogen (200). Gaebler and Robinson (215) conclude from their studies that the presence of insulin facilitated the nitrogen retention by growth promoting extracts, but that it was not essential for this process. It is difficult to determine from available data whether the effects observed are due to the primary influence of a particular hormone, or whether they are due to alterations in the endocrine balance of those hormones concerned with nitrogen metabolism (200). This conclusion applies to the observations on the metabolic effects of epinephrine which is believed to act indirectly through the adrenotrophic hormones (216).

Adrenocorticotrophin and Adrenal Steroids

The effect of stress (219, 220, 221, 222, 159, 223) and of the adrenal and its regulatory hypophyseal hormone, adrenocorticotrophin, on nitrogen metabolism is well known (224, 225). The pituitary-adrenal system, which has been reviewed by Sayers and Sayers (216), and its relationship to stress (226) has received considerable attention in recent years, especially since the isolation of adrenocorticotrophin (227, 228) and the synthesis of active adrenal steroids by Reichstein (229) and Kendall (230).

Harrison and Long (231) confirmed the observations of Evans in 1936 (232) that adrenalectomized rats excreted about 25% less nitrogen than did normal rats under similar conditions.

Subsequently, Long and his co-workers (233) showed that the administration of cortical extracts or active adrenal crystalline steroids, to either fasted normal or adrenalectomized mice and rats, is followed by a large increase in liver glycogen and a slight hyperglycemia. There was also an increase in nitrogen excretion of sufficient magnitude to suggest that the increased protein catabolism is the source of this newly formed carbohydrate. As a result of these observations the authors postulated that one of the properties of the cortical hormones, or so called "sugar hormones", is the stimulation of protein catabolism and gluconeogenesis (234). Subsequent work led to the suggestion that the adrenal steroids acted by interference with oxidation of glucose in the peripheral tissues (234, 235, 236). Kendall (234) administered compound E to an adrenalectomized, pancreatectomized dog and found that the first effect was an increased glycosuria which was not accompanied by an increased nitrogen output. These observations led Ingle (237) and Drury (238) to suggest that the inhibition of carbohydrate utilization may cause the increased gluconeogenesis, on the basis of the observed increased protein catabolism in pancreatic diabetes, phlorhizin diabetes and starvation. That this was not likely was shown by Ingle. He found that following the administration of adrenal steroids the increased nitrogen excretion preceded the glycosuria, and that insulin failed to prevent the increased nitrogen excre-

tion (239). The demonstration by Dougherty and White (240, 241) of dissolution of lymphoid tissues and lymphocytes with the pituitary and adrenal hormones would seem to suggest that the primary effect of these hormones is on the tissue proteins. It, however, does not preclude the possibility of a primary anti-insulin effect in the inhibition of carbohydrate utilization. This was admitted by these workers, despite observed increases in antibody titer following administration of these hormones (242).

There has been much speculation regarding the exact nature of the protein catabolic responses to the adrenal hormones. The possibilities that their effect might be due to a degradation of protein, deamination of amino acids, conversion of three carbon precursors to glucose and glycogen, the inhibition of glucose utilization (235) and to an anti-anabolic effect (243) have all been considered.

Observations and studies by Albright in Cushing's Syndrome, in which there is overactivity of the adrenal cortex and a deficiency of tissues, led to the postulation that the effect of these hormones may be due to an inhibition of protein synthesis, or anti-anabolism as suggested by Reifenstein (243). It was felt that this hypothesis would explain the development of ketosis in Cushing's Syndrome, the observed failure to go into negative nitrogen balance, the alimentary glycosuria and the absence of liver glycogen and glucose on a low nitrogen intake. These observations,

Albright felt, could not be fully explained by his previous hypothesis of hypergluconeogenesis and resistance to glucose oxidation.

The experiments of Engel et al (244, 245), who studied the rate of urea formation in fasted nephrectomized rats treated with adrenal cortical extract, showed an increase in urea formation which was inhibited by intravenous glucose. Injections of the essential amino acid mixture failed to produce an increase in urea greater than the amount observed when either the amino acid mixture or adrenal extract was given alone. This suggested to the authors that the amino acids actually inhibited the breakdown of endogenous protein which usually followed the injection of the adrenal extract in fasted animals. They found, however, if rat albumin were given intravenously, there was a greater increase in the amount of urea formation when the extract was given, and that this effect was also abolished if glucose were given with the albumin. It was accordingly suggested that the adrenal cortex is necessary to make protein available for catabolism, but only when no other substances capable of entering the metabolic pathway of carbohydrates are available. It was further postulated that this action of the adrenal cortical hormones is on the whole protein rather than the amino acids.

Further evidence for this mode of action was obtained in the studies of blood amino acid levels. It has been shown that

there is a fall in the blood amino acid level in the eviscerated adrenalectomized rat (246, 247) and in the non-eviscerated adrenalectomized rat (248), as compared with the non-adrenalectomized controls. Furthermore, treatment of the eviscerated adrenalectomized rat with adrenal cortical extract (247) and cortisone (249) caused significant increases in the plasma amino acid concentration. Ingle obtained an average increase of 60% in his study which was found not to be due to hemoconcentration (249), and accordingly postulated that the changes observed represented an extrahepatic effect of cortisone, since the liver was absent. Clinical data obtained from patients treated with ACTH confirm these observations of an increased blood amino acid (250, 251).

Other workers have obtained evidence conflicting with the above interpretations and have suggested that the hormones act at the amino acid level (252, 253). Cagan reported (252) that the amino acid oxidase activity of various tissue homogenates from adrenalectomized animals was less than in normals, and could be restored following treatment with adrenocortical extract. Furthermore , casein hydrolysate given to normal rats caused an increase in amino acid oxidase activity of tissue homogenates. This was not observed in adrenalectomized animals, in which there was a delayed clearance of amino acids from the blood, together with lesser increases in blood urea and glucose than in the normals. Engel studied the effect of the administration of amino acids on the rate of urea formation in nephrectomized,

adrenalectomized animals and in similar rats treated with cortisone (253). He found that in the former animals the hourly rate of urea formation after intravenous injections of amino acids was decreased during the first hour, but there was no difference in the second hour. The decrease, however, was not accompanied by a delayed fall in blood amino acids. It was furthermore noted that intravenous glucose significantly depressed the amount of urea formed 1 to 2 hours after the injection of amino acids in both the control nephrectomized rats and in the adrenalectomized nephrectomized rats. This was not observed in the ACTH treated nephrectomized ones. However, the blood amino acids were significantly higher in the former two groups, but not in the latter. These observations led Engel to suggest that the adrenal cortex exerts an influence on the amino acid catabolism in the liver as well as on the protein in the periphery, and that this former effect could only be demonstrated when large amounts of glucose were given.

Studies of the effects of the adrenal steroids and adrenocorticotrophins on nitrogen metabolism by means of isotopes have confirmed these observations, but have not offered further clarification of their mode of action. Incorporation of labelled amino acids into tissues was found to be more rapid in adrenalectomized animals than in normals (254), and with adrenal steroid treatment the rate of incorporation was depressed (255). Similar observations were made in the hypophysectomized rats treated with ACTH, in which there was an increased uptake of the isotope by the

tissues (256). Recently, an anti-anabolic effect of cortisone was reported on the basis that labelled antibody did not disappear from the serum any faster than in the untreated controls (298).

Parson carried out similar studies in humans using glycine labelled with N 15 in the diet (266). Following the administration of ACTH there was noted an increased excretion of the N 15 in the urine, similar to that obtained in a case of Cushing's Syndrome. He suggested that this might represent a decreased anabolism of protein.

The introduction of methods for measuring adrenal cortical function has led to more correlative studies on nitrogen metabolism in various disease and traumatic states. Observations on various clinical conditions, carried out by Browne and co-workers (1), showed that while there is often a correlation between increased nitrogen excretion and adrenal cortical activity in stress, as measured by urinary corticoids, this is not always so. Cases were reported in which the urinary nitrogen was not increased despite high urinary corticoids. It was also shown that a patient may have normal corticoids in the presence of injury and infection and still be capable of storing large amounts of nitrogen. Browne stressed the fact that the nitrogen responses depend not only on the amount of adrenal hormones produced, but also on the total metabolic status of the body. Such factors as the varied responses to stress resulting from failure of the hypothalamus, the pituitary and the adrenal, as well as the amount

of adrenal hormones produced relative to the needs of the body under specific circumstances, were considered to result in altered metabolic reactions. Moreover, the response to the adrenal hormones was shown to be dependent on the general metabolic status of the body (1).

The animal experiments of Munroe, Cuthbertson and others (258, 259, 260, 257) have also shown that the response to injury will depend on the previous nutritional state. Following fracture in the fed rat there was increased nitrogen excretion (259), but not in the protein depleted ones (260). Similar observations have been made in patients in their response to injury (1, 261). That these responses can be altered by the nitrogen sparing effect of fat and carbohydrates have also been demonstrated in animals (262, 263) and humans (264, 222, 265). Mackenzie has presented evidence to show that the protein sparing effect of carbohydrate is not mediated through the adrenal (297).

Consideration of these observations has led Browne to speculate on a possible mechanism of action of the adrenal steroids which might explain the various responses in nitrogen metabolism, as measured by the urinary nitrogen excretion (1). It has been pointed out that there may be nitrogen equilibrium in the presence of abnormal nitrogen metabolism. This is in accord with the findings of Parson (266) who fed N 15 glycine to normal subjects receiving ACTH, and to patients with Cushing's Syndrome receiving testosterone. It was found that though they were in nitrogen equilibrium,

there was an increased excretion of the N 15 in the treated normals up to the levels seen in Cushing's Syndrome, and that testosterone lowered the high excretion in Cushing's Syndrome.

It was postulated by Browne that the adrenal hormones exert their effect on the internal body sources of protein by altering homeostatic mechanisms in such a way as to render them more mobilizable in times of emergency (1). This could be compared to the catabolic or anti-anabolic theory of the "S" hormones of Albright (243). However, this failed to explain the low nitrogen excretion in the presence of increased adrenal cortical activity, as indicated by the increased urinary corticoid excretion, and which has been observed in pregnancy and chronic disease states. This, Browne believed, was due to the presence of a strong anabolic focus in the body which utilizes the protein mobilized by the activity of the adrenal cortical hormones (1). This would seem to explain the observed lack of correlation between nitrogen excretion and adrenal cortical activity, for if the anabolic focus were relatively small there would be increased excretion, and if large enough there would be the expected decreased nitrogen excretion. That these internal transfers of body protein via the plasma may be possible, appears valid in view of Whipple's theory that body proteins are in a state of dynamic equilibrium in which tissue and plasma proteins are freely exchangeable (2, 3).

In view of this concept, one should expect to be able to

demonstrate an alteration in plasma protein levels following the administration of the ACTH and adrenal cortical hormones, since its effect should be mediated via the plasma proteins. Plasma protein depletion would also, according to this theory, create a strong anabolic focus which should be ideal for the study of internal transfers of protein under the influence of these hormones (267).

THE EFFECT OF THE PITUITARY AND ADRENAL HORMONES ON PLASMA PROTEINS

The experimental studies directed towards an understanding of the relationship of pituitary and adrenal hormones to the plasma proteins have yielded many conflicting reports (268). This is not unexpected, since the use of experimental procedures such as hypophysectomy and adrenalectomy are accompanied by marked disturbances of many of the homeostatic mechanisms of the body.

Goldberg (269, 270) observed that following hypophysectomy in dogs there was a fall in serum albumin and a rise in the globulin. Desiccated thyroid, however, caused a return of the globulin fraction to normal levels, but had no effect on the albumin fraction. This led the author to suggest that the decreased thyroid activity following hypophysectomy was responsible for the changes in serum protein levels. Similar observations in the monkey (271) and rat (272) confirmed the above findings. Levin and Leathem noted that the albumin and globulin changes following hypophysectomy were not solely due to the inanition accompanying the procedure, and that thyroidectomy alone caused an increase in serum globulin with little effect on the albumin level (272, 273). Treatment of the hypophysectomized rats with thyroxine prevented the increase of globulin without inhibiting the decrease in serum albumin (272). However, adrenal cortical extract or desoxycorticosterone prevented the fall of

albumin, but had little or no effect on the globulin level. These findings led the authors to conclude that the serum albumin level was maintained under adrenal cortical influence, and that serum globulin was associated with thyroid activity.

Subsequently, studies in adrenalectomized cats (274) demonstrated a rise in the total protein which was mainly due to a globulin increase, since despite the hemoconcentration the serum albumin concentration tended to decrease or remain at the normal levels. Following treatment with ACE or DCA there was hemodilution with a corresponding decrease of serum globulin and total protein. The serum albumin level, however, rose. Other workers (275) confirmed these findings in adrenalectomized rats and cats, but found that the globulin and albumin-globulin ratio remained elevated despite replacement therapy. It was considered, accordingly, that the adrenal is important in the maintenance of normal levels of globulin as well as albumin.

These observations of increased serum globulin and decreased albumin-globulin ratios following hypophysectomy have been confirmed by electrophoretic studies (276, 277), and have led to investigation of the possible role of the thyroid in globulin formation. Electrophoretic analysis of the elevated globulin in the serum of hypophysectomized rats has revealed the presence of an alpha globulin which was not present in the normal rats, and which was considered to be due to the absence of the thyroid (277). That this may be possible was shown by the rise

of the globulin fraction following the administration of thiourea (278). It has been shown, however, that injuries and diseases in man (279) and in dogs (284) cause a rise in the alpha globulin fraction. This would suggest that possibly the trauma associated with hypophysectomy was responsible for the rise, rather than the absent thyroid. Li and Reinhardt administered ACTH to normal and hypophysectomized rats and found a rise in the plasma albumin concentration in both, but no increase in the globulin fraction (280).

The studies of Dougherty and White on the effects of the pituitary and adrenal hormones on lymphoid tissue has directed attention towards the primary effect of these hormones being on the globulin, rather than the albumin fraction. They have demonstrated in mice a decrease in lymphoid tissue following the administration of ACTH (281), an observation which has been confirmed in rats (282). A marked decrease in the absolute number of lymphocytes has also been observed (283, 241). Sayers, White and Long (228) have produced alterations in the histology of the lymphoid tissues in one to two hours after the administration of 1 mgm. of ACTH, and in three to six hours there was noted a marked depletion of the lymphocytes in the lymphoid tissues. These changes did not occur in the adrenalectomized animals (282, 283, 240, 241) unless they were treated with adrenal cortical extract, corticosterone or compound "F" (241). These observations offer additional supportive evidence for the adrenal mediation of the

pituitary adrenocorticotrophic hormones.

Since the involution of the lymphoid tissues and the lymphopoenia were observed to occur at the same time a serum protein increase of 0.3 to 0.5 grams per 100 ml. was noted, Dougherty and White suggested that these are the sources of the protein and that they are under the control of the pituitary and adrenal hormones (240). They furthermore noted that the agglutinin titers to sheep red blood cells are enhanced in rabbits by either prolonged or single injections of pituitary adrenotrophic and adrenal cortical extracts (242). This enhancement of antibody function has been also demonstrated in mice and rats following administration of adrenal cortical extracts (299). Others, however, have failed to confirm this (285). The above observations led the authors to assume that the increased plasma protein belonged in part to the blood proteins containing antibody, namely, the globulins (286). Following single or repeated injections of ACTH in rats a rise in the total serum proteins was observed, while adrenalectomy caused a fall. It was also noted that the administration of ACTH or adrenal cortical extract to rabbits produced an increase in the concentration of total serum globulin which was due to significant increases in the beta and gamma globulin fractions (286). In keeping with this concept, it has been shown by the electrophoretic analysis of lymphoid tissue extracts that they contain a fraction with the motility identical with gamma globulin of normal rabbit serum (286, 61).

Although the hypothesis of Dougherty and White is very interesting and appeared to be supported by their experimental studies, attempts to confirm these alterations in the plasma proteins, following the administration of adrenal cortical extracts in mice (287), dogs (288) and humans (289, 290, 291, 288), have failed. Milne and White were able to obtain an increase in the total protein, but were unable to find any significant rise in the globulin fraction by electrophoretic studies (287). Recent investigation of patients treated with cortisone and ACTH has revealed a depression of the gamma globulin and fibrinogen levels (293, 294), although measurement of the total circulating protein showed changes in the opposite direction. Electrophoretic analysis of the plasma proteins of patients with Cushing's Syndrome showed lowered albumin and gamma globulin levels, but no change in the total protein (295). Leutcher, in a study of Addison's disease, found that the total protein was in the high normal range, with the albumin significantly decreased and the globulin increased (279). Observations by McCullagh in Addison's disease showed a lowered serum albumin which increased following treatment with ACE or DCA (296).

The apparent confusion and lack of accord in the literature regarding the effect of these pituitary and adrenal hormones on the plasma proteins would seem to warrant more careful studies of their effects on the total circulating protein pool. It is apparent that without these studies alterations in the

plasma protein might be easily masked if concentrations alone are measured. In the experiments which follow, an attempt was made to measure the absolute amount of circulating protein before and after depletion induced by a single massive hemorrhage, and to determine the effect of these hormones on their regeneration.

EXPERIMENTAL METHODS

General

The effect of ACTH on the regeneration of plasma protein was investigated by measuring the total amount of circulating protein regenerated in the rat following a single large withdrawal of blood. The plasma volume before and after hemorrhage was measured by the washing out and perfusion technique, described in detail by Cutting and Cutter (67, 183).

The animals used in these experiments were male hooded rats obtained from the colony maintained at this laboratory. The rats were allowed ad libitum quantities of water and the standard diet of Purina Fox Chow and bread.

In the initial six experiments rats weighing approximately 250 grams were selected randomly from the colony. In the subsequent group, the rats to be used in the experiment were placed in a separate cage from the rest of the colony and allowed to gain weight up to 250 grams while on a similar diet. Of these, only animals which showed normal weight gain were used in order to assure as normal a nutritional state as possible.

The operative technique allowed experiments to be performed on only one pair of rats at a time, one control and one treated. The ACTH used was a crystalline extract obtained from the Armour laboratories under the name of "ACTHAR". This was dissolved in saline and a dose of 4 mgms in a volume of 0.5 ml. was

injected intramuscularly into the femoral muscles.

Operative Procedures

The rats were anaesthesized with ether, weighed and secured in the dorsal position. The head was hyperextended and overhanging the edge of the operating board. The right jugular vein was dissected free and the entire head, as well as the wound, was covered with vaseline. Two ligatures were placed loosely around the vein and a drop of a saturated solution of sodium citrate from a tuberculin syringe was dropped on the vein between the two ligatures. The proximal ligature was tied and the vein nicked distally, allowing the blood to run directly into a graduated centrifuge tube. The animal was bled approximately 35% of its blood volume which, in the size of rats used, was about 5 ml. Citrate was constantly dropped on the bleeding vein to prevent clotting.

It was found convenient, in order to avoid spillage, to cut the skin in such a way as to form a tunnel for the blood dropping into the centrifuge tube. The citrate was dropped from a 30 gauge needle connected to the syringe in order to allow a uniform mixing with the blood, and to limit the amount of anti-coagulant used to 0.05 ml. When the necessary amount of blood was removed the distal ligature was tied. The vaseline on the exposed area of the dependent head allowed complete collection of the blood sample without leaving any appreciable amounts of blood on the tissues. The wound was then closed with skin clips.

The animal was maintained under ether anaesthesia throughout the procedure and, after closing the wound, was injected with 4 mgms of ACTH in 0.5 ml. saline. The control was given an equal volume of saline. There was rapid recovery without any mortality and during the 5½ hour period before the final bleeding, only water was allowed.

The animal was anaesthesized again and the left jugular vein exposed. The distal end of the vein was ligatured and the proximal end cannulated with an 18 gauge polyvinyl plastic tubing, directed towards the heart. This was secured with another ligature. The diameter of the cannula could be adjusted to the size of the vein by warming and stretching. The tubing, which was connected to a bottle of normal saline, was of sufficient length to allow some movement of the head without danger of slipping out of the vein. Loss of blood was prevented during the cannulation by pulling on the proximal untied ligature. The right jugular vein was exposed again by removing the skin clips following which it was cut distally to its ligature. Another specimen of blood of approximately 4 to 5 ml. was collected in a centrifuge tube and a slow infusion of saline was started in the other vein. The perfusate from the bleeding jugular vein, which was washed continuously with isotonic citrate, was collected in a beaker.

Care had to be exercised to prevent any loss of blood during both procedures. The rate of the perfusion was controlled to avoid any overloading of the circulation in order to maintain

maximum cardiac function sufficiently long enough to exsanguinate the animal as completely as possible. This was indicated by the perfusate becoming clear. The rate was found to be usually about 20 to 30 drops a minute, depending upon the bore of the cannula. A quantity of 50 to 75 ml. of saline was usually required to complete the perfusion.

Maintenance of cardiac action, by controlling the perfusion rate, was found to be necessary to effect satisfactory exsanguination, otherwise pooling of the blood in the veins of the viscera and extremities was noted. The completeness of perfusion was determined by examining the peripheral and abdominal veins which were usually found to be free of blood. Small amounts, however, were invariably noted in the coeliac vessels, due to the impossibility of removing all the blood from the spleen. This amount was quite constant and considered negligible, since the addition of this blood to the perfusate did not significantly affect the measurement of the amount in the original perfusate. This was determined by washing the cut inferior vena cava and measuring the amount of blood obtained from this vessel and its branches. Hematocrit and volume measurements of the perfusate, before and after addition of the remaining quantity of cells, did not reveal any significant measurable differences in the volumes of the packed red cells.

In some cases the exsanguination was unsatisfactory due to early cessation of cardiac action and subsequent incomplete perfusion. This was readily determined by examining the abdominal

and peripheral veins in all animals following the perfusion. In such cases of incomplete perfusion, the animals were discarded and the experiment repeated. The experiment was repeated also in all cases where there was any loss of blood, or where there was clotting or hemolysis of the specimens.

Calculation of the Plasma Volume

The volume and hematocrits of the two blood samples and of the perfusate were determined in duplicate. From these, plasma volumes before and after the hemorrhage were calculated. This was simply done on the basis of the ratios of plasma volume to packed red cell volume, according to the following formulae.

Plasma volume before hemorrhage $= \frac{A \times B}{C}$, where

A is the sum of the packed red cell volume of the perfusate and the first and second blood samples,

B is the plasma volume of the first blood sample,

C is the packed red cell volume of the first blood sample.

Plasma volume after hemorrhage $= \frac{X \times Y}{Z}$, where

X is the sum of the packed red cell volume of the perfusate and the second blood sample,

Y is the plasma volume of the second blood sample,

Z is the packed red cell volume of the second sample.

Chemical Methods

Total plasma protein and blood non-protein nitrogen were determined by a micro-modification of the standard Kjeldahl proce-

dure, and all determinations were done in duplicate.

1. Reagents

A. Acid Digestion Mixture

i) 8 grams selenium dioxide
ii) 8 grams copper sulfate
iii) 600 ml. nitrogen free concentrated sulfuric acid

These were mixed and heated to boiling in a large flask, with excessive fuming prevented by covering the mouth of the flask with a small funnel.

B. Indicators

i) 15 ml. of 1% aqueous Methyl Green solution
125 ml. of 2% Methyl Red solution in 95% alcohol
ii) 0.5% alcoholic solution of Phenolphthalein

C. N/14 Sulphuric Acid

D. 40% Sodium Hydroxide

E. 2.5% Solution of Boric Acid

F. 0.86% Sodium Tungstate

G. 2/3 Normal Sulphuric Acid

All were prepared according to standard chemical methods.

2. Procedure for N.P.N. Determination

Preparation and Digestion: The cells were laked and the protein precipitated by delivering a 0.2 ml. sample of whole

blood into a centrifuge tube containing 3.5 ml. of a 0.86% solution of sodium tungstate, to which was added 0.3 ml. of 2/3 N sulphuric acid, according to the method described by King (300). The sample was centrifuged for 15 minutes and 1 ml. of the supernatant was then added to 1 ml. of the sulphuric acid digestion mixture in a 10 ml. pyrex micro-Kjeldahl flask. One ml. of the supernatant was then equivalent to 0.05 ml. of whole blood. Sandstones were added to the sample to prevent bumping and the flask placed in a sand box, heated by a series of small Bunsen burners. The neck of the flask was loosely stoppered by a sealed glass bulb to prevent any loss during heating. Digestion was complete in about 15 minutes as indicated by the solution turning colorless.

Distillation: This was carried out in a steam jacketed distillation apparatus described by Markham (301). He found it suitable for analysis of quantities of nitrogen as low as 0.02 mgm.

The sample was alkalinized with 5 ml. of 40% sodium hydroxide containing a drop of phenolphthalein as an indicator. The ammonia was distilled into a wide mouthed test tube containing 1 ml. of 2.5% boric acid and 0.01 ml. of the indicator mixture of methyl red and methyl green.

Prior to use the Markham apparatus was steamed out, and blank determinations were made on identical reagents and quantities, except that distilled water was used instead of the

nitrogen sample. These blanks varied considerably from day to day and were accordingly subtracted from the titration value of the analyzed sample.

Titration: This was carried out with a micro-burette which was especially suitable for measuring small amounts of nitrogen. The burette used was described by Sholander (302, 303) who reported an accuracy corresponding to one part in 10,000 of the total delivery capacity of the burette. This has been confirmed in this laboratory by Mackenzie (297) who constructed the burette used in these experiments.

The burette operates by movement of a commercial micrometer spindle which is accurately divided into 2,500 parts. This displaces mercury, forcing the titrating liquid from a very fine gauged burette. The working capacity of the assembled burette was found to be 0.495 ml., with one micrometer division corresponding to 0.00062 ml. of the titrating solution, which in this experiment was 1/14 N sulphuric acid. Accordingly, one micrometer division corresponded to 0.00062 mgm of nitrogen.

The titration was carried out by immersing the tip of the burette into the distillate through which nitrogen gas was bubbled to facilitate mixing with the sulphuric acid. The reading was taken directly from the micrometer spindle with the approximate correction made for the blank. The N.P.N. was then calculated.

All analyses were carried out in duplicate and they were repeated when there was any marked difference between the results of the duplicate samples.

3. Procedure for Plasma Protein

The plasma protein nitrogen was determined by a similar micro-Kjeldahl procedure.

0.2 ml. of plasma was diluted with 3.8 ml. of saline and thoroughly mixed. One ml. of the mixture was digested with 1.5 ml. of the sulphuric acid mixture, and the sample then distilled in the Markham apparatus as described above, after alkalinization with 8 ml. of 40% sodium hydroxide. Titration was carried out with 1/14 N sulphuric acid, using a micro-burette graduated in 0.01 ml.

The number of milligrams of plasma protein nitrogen in the sample equalled numerically the volume of the acid titrated after correction for the blank. The plasma protein was then calculated by applying the dilution factor, and the factor 6.25 for conversion of the nitrogen to protein.

RESULTS AND DISCUSSION

To facilitate interpretation of the results, a few general introductory remarks may be made about the method. Although attempts were made to use rats weighing 250 grams, this was often impossible. It will be noted in Table I, however, that each member of the pair used in an experiment was approximately the same weight, with usually not more than a 2 to 4 gram difference. The mean weights of the rats in each series of experiments showed only a 1 gram difference between the control and treated animals.

In attempting to standardize the experiments further, it was considered important to bleed the animals of identical quantities of blood. This was technically impossible and approximately 4.5 - 5.0 ml. were removed which, according to the calculations, represented 30 - 45% of the initial blood volume. The mean percentage difference removed between the control and treated animals did not show as wide a variation. It will be noted in Tables I and II that the difference was 3.5% in Series I and 0.3% in Series II. However, because of the variation in the amount of blood removed in the individual experiments, the recovery of total circulating protein and plasma volume was measured as the percentage difference between that amount remaining immediately after hemorrhage and the final amount, both expressed as a percentage of the initial determination.

The washing-out and perfusion technique for measuring plasma volume in rats is a simple one in that it obviates the use of a micro-chemical dye method. Furthermore, the measurements by Cutting and Cutter (183) have compared favorably with the results of Metcoff, Cutting and Favour (176) who have used the dye method. However, the technical aspects of the washing-out and perfusion procedure are fraught with many time consuming difficulties which necessitate repetition of the experiments. This limited the number which could be carried out. The problems most frequently encountered were incomplete exsanguination, due to premature death of the animal, and marked variations in the duplicate hematocrits. Since the measurement of the plasma volume was dependent on the total red cell volume and the hematocrit, it is obvious that any such inaccuracies would necessitate repetition of the experiment. Although every effort was made to control these sources of error, this was not always possible.

Another important factor which must be considered is the variable amount of red cells which is added to circulation by the spleen following hemorrhage. This would tend to increase the initial calculated total packed red cell volume, which in turn would give a high value for the plasma volume and the total circulating plasma protein before exsanguination. Thus the total amount of protein regenerated, as well as the volume recovery, would be lower than its true value. It is apparent that variations in the size of the red cell will alter the hematocrit and conse-

quently the plasma volume.

With respect to the collection of the blood samples for chemical examination, hemolysis occurred frequently. This was overcome to a great extent by carefully covering the exposed operated area with vaseline, thus preventing the tissue fluids from mixing with the blood sample. Such admixture would also tend to lower the hematocrit. Despite these precautions small amounts of hemolysis were unavoidable and could not be accurately measured. This occurred infrequently and was never greater than 0.1 gram per 100 ml.

The Effect of ACTH on Plasma Volume

Tables I and II show the plasma volumes before and 5½ hours after depletion in the experiments of Series I and II respectively. It should be noted that there is a marked variation in the initial volumes even in animals of the same weight. However, the mean initial plasma volumes of the control and of the treated animals in Series I and II showed a variation of less than 1 ml. There was also a wide variation in the final volumes, but here again the variation in the mean values of the control animals in each series was 0.5 ml. and of the treated animals in Series I and II, less than 1 ml. The percentage volume recovered varied considerably. Four of the 7 animals in Series I,and 6 of the 8 animals in Series II showed a greater recovery in the treated group than the controls, as shown on the extreme right of Tables I and II. On comparing the percentage recovery it will be noted that in each

PLASMA VOLUMES BEFORE AND AFTER HEMORRHAGE

SERIES I

Controls

Rat Pair No.	Rat Wt. (gms)	Initial Plasma Volume				Final Plasma Volume		
		Initial Volume (ml.)	Volume Removed (ml.)	Percent Removed	Percent Remaining	Final Volume (ml.)	Percent of Initial Volume	Percent Volume Recovered
1	242	8.37	2.82	33.6	66.4	7.62	91.0	24.6
2	252	9.64	2.91	30.2	69.8	9.93	103.0	33.2
3	261	8.85	2.84	32.2	67.8	9.17	103.7	35.9
4	246	7.39	2.41	32.6	67.4	8.47	114.8	47.4
5	254	7.60	2.79	36.7	63.3	7.75	102.0	38.7
6	250	7.84	2.98	38.0	62.0	7.97	101.8	39.8
7	253	6.37	2.46	38.7	61.3	8.55	134.2	72.9
MEAN	251	8.01	2.74	34.6	65.4	8.49	107.2	41.8

Treated

1	241	8.28	2.97	35.9	64.1	8.48	102.3	38.2
2	251	6.75	2.35	34.9	65.1	8.35	123.7	58.6
3	259	6.60	2.55	38.6	61.4	7.95	120.4	59.0
4	250	7.60	2.81	37.1	62.9	8.22	108.2	45.3
5	253	6.56	2.99	45.7	54.3	6.85	104.5	50.2
6	254	8.30	2.97	35.9	64.1	7.73	93.2	29.1
7	249	6.84	2.65	38.8	61.2	8.38	122.4	61.2
MEAN	251	7.28	2.76	38.1	61.9	7.99	110.7	48.8

TABLE I

PLASMA VOLUMES BEFORE AND AFTER HEMORRHAGE

SERIES II

Controls

Rat Pair No.	Rat Wt. (gms)	Initial Plasma Volume				Final Plasma Volume		
		Initial Volume (ml.)	Volume Removed (ml.)	Percent Removed	Percent Remaining	Final Volume (ml.)	Percent of Initial Volume	Percent Volume Recovered
8	249	6.30	2.70	42.8	57.2	6.47	102.7	45.5
9	250	7.33	2.67	36.4	63.6	7.28	99.4	25.8
10	254	8.36	2.63	31.4	68.6	8.89	106.4	37.8
11	250	8.79	2.91	33.1	66.9	7.02	79.8	12.9
12	253	7.94	2.90	36.5	63.5	8.83	111.2	47.7
13	237	8.40	2.87	34.2	65.8	8.92	106.2	40.4
14	234	6.67	2.59	38.9	61.1	7.49	112.3	51.2
15	245	7.65	2.57	33.6	66.4	8.75	114.4	48.0
MEAN	246	7.68	2.73	35.8	64.1	7.96	104.1	38.7

Treated

8	253	7.47	2.79	37.3	62.7	9.13	122.1	59.4
9	247	7.07	2.91	41.2	58.8	8.22	116.3	57.5
10	253	8.56	2.88	33.6	66.4	8.95	104.6	38.2
11	246	9.52	3.16	33.2	66.8	9.15	96.2	29.4
12	247	8.37	2.95	35.2	64.8	9.99	119.3	54.5
13	233	7.67	2.77	36.1	63.9	8.01	104.5	40.6
14	232	7.57	2.70	35.6	64.4	8.12	107.3	42.0
15	248	8.55	3.12	36.5	63.5	9.18	107.4	43.9
MEAN	245	8.10	2.91	36.1	63.9	8.84	109.7	45.8

TABLE II

series the treated animals recovered, on the average, 7% more of their plasma volume than did the controls. This is merely suggestive, but not conclusive evidence, that there is an increase in the plasma volume of the animals treated with ACTH.

The Effect of ACTH on the N.P.N. and Plasma Protein Concentration

Tables III and IV show the plasma protein and non-protein nitrogen concentrations before and 5½ hours after plasma depletion. The recovery in the plasma protein and the change in the N.P.N. were both determined by the final concentration expressed as a percentage of the original concentration. Here again there was a considerably wide range of recovery in both the control and treated animals, as well as in the initial concentrations.

The recovery of the plasma protein was slightly greater in the treated group in 5 of the 7 animals in Series I, and 6 of the 8 animals in Series II. The differences in the mean values between the control and treated groups in the two series were 1.5% and 1.6% respectively (Table III).

The non-protein nitrogen showed a more marked variation, with however, a higher percentage change in the treated group in 5 of the 7 animals in Series I, and 6 of the 8 in Series II. The differences in the mean values of the two series were 4.7% and 7.5% respectively. The variations in the final non-protein nitrogen is to be expected, in view of the possible disturbances in kidney function following massive hemorrhage.

These results again seem to indicate only that there may

PLASMA PROTEIN CONCENTRATION BEFORE AND AFTER HEMORRHAGE

SERIES I

EXP. NO.	CONTROL			TREATED		
	Initial Conc. (gms/100 ml.)	Final Conc. (gms/100 ml.)	Percent of Initial Conc.	Initial Conc. (gms/ 100 ml.)	Final Conc. (gms/ 100 ml.)	Percent of Initial Conc.
1	6.50	5.63	86.0	6.45	5.69	88.3
2	5.95	6.13	102.9	5.86	5.57	95.3
3	5.79	5.58	96.3	6.01	6.00	99.9
4	6.99	6.08	86.9	6.43	5.84	90.9
5	6.52	5.84	89.6	6.53	5.84	89.5
6	6.35	5.59	88.1	6.20	5.95	96.1
7	6.31	5.50	87.3	6.72	5.93	88.2
MEAN	6.34	5.76	91.1	6.31	5.83	92.6

SERIES II

8	6.55	5.43	82.8	7.04	5.51	78.3
9	6.69	6.06	90.7	6.57	5.69	86.7
10	6.42	5.46	85.0	6.18	5.69	92.3
11	6.19	5.98	96.6	5.98	5.89	98.7
12	6.56	5.76	87.8	6.01	5.45	90.7
13	6.21	5.73	92.3	6.10	5.86	96.2
14	6.55	5.94	90.8	6.44	6.06	94.1
15	6.73	6.15	91.4	6.36	5.95	93.7
MEAN	6.49	5.81	89.7	6.33	5.76	91.3

TABLE III

NON-PROTEIN NITROGEN CONCENTRATION

BEFORE AND AFTER HEMORRHAGE

SERIES I

Exp. No.	CONTROL			TREATED		
	Initial Conc. (mgms/ 100 ml.)	Final Conc. (mgms/ 100 ml.)	Percent of Initial Conc.	Initial Conc. (mgms/ 100 ml.)	Final Conc. (mgms/ 100 ml.)	Percent of Initial Conc.
1	54.6	63.9	117.2	50.5	62.6	123.8
2	52.1	55.8	107.1	84.4	58.0	68.8
3	52.7	51.4	97.2	48.0	58.3	121.3
4	72.5	92.3	127.3	66.3	81.2	122.7
5	63.2	57.6	91.2	54.0	57.6	106.6
6	66.3	52.0	78.4	53.3	47.7	89.6
7	54.6	58.3	106.6	57.0	71.3	125.0
MEAN	59.4	61.6	103.6	59.1	62.4	108.3

SERIES II

8	72.0	67.6	93.8	72.6	69.8	96.3
9	56.4	67.0	118.7	53.6	60.7	113.4
10	50.3	37.8	75.3	47.7	56.1	88.3
11	56.4	48.7	86.3	57.7	44.6	77.3
12	61.4	52.1	84.8	48.4	53.3	111.0
13	44.6	45.2	101.6	46.5	54.5	117.3
14	55.2	56.1	101.6	59.5	60.7	102.2
15	75.0	48.3	64.3	60.8	49.0	80.7
MEAN	58.9	52.8	90.8	55.8	54.8	98.3

TABLE IV

be a greater increase in the plasma protein and non-protein nitrogen concentrations in the treated animals as compared with the controls.

The Effect of ACTH on the Total Circulating Protein

Since the treated animals have tended to show greater recovery in the plasma volume and plasma protein concentration, it is apparent that an increase in the total circulating protein would also be found. The results of the initial and final total circulating protein are shown in Tables V and VI. These have been expressed in absolute values, since measurements of the percentage recovery in terms of unit of surface area and also of body weight were not different from those obtained from the absolute values.

It will be noted that the majority of the treated animals showed a greater recovery of the total plasma protein than did the untreated controls, in both series of experiments. In Series I the treated group demonstrated an average recovery of 102.6% of the initial amount present, as compared with 97.4% for the control group. The values in Series II were similarly 99.7% and 93% respectively.

Since the amount of protein which was removed varied in each animal, the recovery was measured also by the difference between the total amount of circulating protein remaining immediately after depletion and the final amount. This difference was expressed as a percent of the initial amount of total circulating protein, as

TOTAL CIRCULATING PROTEIN BEFORE AND AFTER HEMORRHAGE

SERIES I

CONTROLS

Rat Pair No.	Rat Wt. (gms.)	Initial Plasma Protein				Final Plasma Protein		
		Initial Amount (gms.)	Amount Removed (gms.)	Percent Removed	Percent Remaining	Final Amount (gms.)	Percent of Initial Amount	Percent Recovered
1	242	.544	.183	33.6	66.4	.428	78.7	12.3
2	252	.574	.173	30.2	69.8	.608	105.9	36.1
3	261	.513	.165	32.2	67.8	.512	99.8	32.0
4	246	.517	.168	32.6	67.4	.514	99.5	32.1
5	254	.496	.182	36.7	63.3	.453	91.3	28.0
6	250	.498	.189	38.0	62.0	.447	89.7	27.7
7	253	.402	.155	38.7	61.3	.470	117.0	55.7
MEAN	251	.506	.174	34.6	65.4	.348	97.4	32.0

TREATED

1	241	.534	.192	35.9	64.1	.482	90.2	26.1
2	251	.395	.138	34.9	65.1	.466	117.8	52.7
3	259	.397	.153	38.6	61.4	.477	120.3	58.9
4	250	.488	.181	37.1	62.9	.481	98.5	35.6
5	253	.428	.195	45.7	54.3	.402	93.7	39.4
6	254	.515	.184	35.9	64.1	.459	89.3	25.2
7	249	.459	.178	38.8	61.2	.496	108.2	47.0
MEAN	251	.459	.174	38.1	61.9	.466	102.6	40.7

TABLE V

TOTAL CIRCULATING PROTEIN BEFORE AND AFTER HEMORRHAGE

SERIES II

CONTROLS

Rat Pair No.	Rat Wt. (gms.)	Initial Plasma Protein				Final Plasma Protein		
		Initial Amount (gms.)	Amount Removed (gms.)	Percent Removed	Percent Remaining	Final Amount (gms.)	Percent of Initial Amount	Percent Recovered
8	249	.413	.177	42.8	57.2	.351	85.0	27.8
9	250	.491	.179	36.4	63.6	.441	89.9	26.3
10	254	.536	.169	31.4	68.6	.485	90.4	21.8
11	250	.544	.180	33.1	66.9	.419	77.2	10.3
12	253	.521	.190	36.5	63.5	.508	97.5	34.0
13	237	.522	.178	34.2	65.8	.511	97.8	32.0
14	234	.438	.170	38.9	61.1	.445	101.8	40.7
15	245	.514	.173	33.6	66.4	.538	104.7	38.3
MEAN	246	.497	.177	35.9	64.1	.462	93.0	28.9

TREATED

8	253	.527	.196	37.3	62.7	.503	95.5	32.8
9	247	.464	.191	41.2	58.8	.467	100.8	42.0
10	253	.528	.173	33.6	66.4	.509	96.5	30.1
11	246	.567	.189	33.2	66.8	.539	95.0	28.2
12	247	.502	.177	35.2	64.8	.544	108.4	43.6
13	233	.468	.169	36.1	63.9	.469	100.3	36.4
14	232	.488	.174	35.6	64.4	.492	101.0	36.6
15	248	.544	.198	36.5	63.5	.546	100.3	36.8
MEAN	245	.511	.183	36.1	63.9	.509	99.7	35.8

TABLE VI

shown in the column at the extreme right of the tables. It will be noted that 5 of the 7 animals in Series I, and 6 of the 8 animals in Series II showed a greater recovery in the treated groups. The mean increase over the controls in the two series was 8.7% and 6.9% respectively.

Comparison of the Effects of ACTH on Plasma Volume, Plasma Protein Concentration, Non-Protein Nitrogen and Total Circulating Protein

The mean percentage recovery of the plasma volume, plasma protein concentration and total circulating protein for the treated and control groups in each series are compared in Table VII. The mean percentage change in the non-protein nitrogen is also shown. All these determinations are noted to be higher in the treated groups, although examination of the values obtained from individual experiments did not show this correlation in all the four measurements. Accordingly, the treated animals which showed an increase or decrease in any one of the measurements did not always show a corresponding increase or decrease in the other three. This is to be expected in view of the many variable sources of error in this experiment which have been previously discussed.

COMPOSITE TABLE OF MEAN PERCENTAGE CHANGES IN PLASMA VOLUME, NPN, PLASMA PROTEIN CONCENTRATION AND TOTAL CIRCULATING PROTEIN

SERIES NO.	PERCENT VOLUME RECOVERED [1]		PERCENT NPN CHANGE [2]		PERCENT PROTEIN CONC. RECOVERED [2]		PERCENT TOTAL CIRC. PROTEIN RECOVERED [1]	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
1	41.8	48.8	103.6	108.3	91.1	92.6	32.0	40.7
2	38.7	45.5	90.8	98.3	89.7	91.3	28.9	35.8

1 See text on Page 66 for explanation.

2 See text on Page 71 for explanation.

TABLE VII

GENERAL DISCUSSION

The subject of plasma proteins has been reviewed as a basis for the consideration of the alteration in nitrogen excretion, following the administration of the pituitary and adrenal hormones. Attempts to define the dynamics of the metabolic changes accompanying their administration have led to conflicting reports in the literature.

The observations of Long (233), Ingle (237), Drury (238) and Engel (244) led them to suggest that the ultimate action of these hormones on protein metabolism was a catabolic one, due either to a stimulation of gluconeogenesis (233) or an inhibition of carbohydrate utilization (234, 235, 236). Albright postulated that the primary action might be anti-anabolic, on the basis of his observations in Cushing's Syndrome (243). The controversial results of the experiments of Dougherty and White have led them to believe that the pituitary adrenocorticotrophic and adrenal hormones acted directly on the lymphoid tissue, causing a release of globulin (240). Further studies have failed to confirm these alterations in the plasma protein fractions (287, 288, 289), although a small increase in the total protein has been found by Milne and White, following the administration of adrenal cortical extract (287).

The present investigation was carried out to test the hypothesis recently presented by Browne (1) who suggested that these hormones might exert their effect by mobilizing or

"loosening" the body protein, thus rendering it available for use in other parts of the body. It was suggested that the plasma protein depleted animal might be particularly suitable for studying this (267), in that the circulating protein pool has been shown to be in a state of dynamic equilibrium with the tissue protein (3, 149) and would be expected to mediate such a proposed effect. Moreover, according to this concept, the plasma would become the anabolic focus as well as the medium for the internal shifts in body protein (267).

It has been demonstrated that specific variations in the total amount of circulating protein becomes evident before the alteration in concentration (181, 183, 184), and that the plasma volume increases or decreases as the total amount of protein increases or decreases. It was accordingly felt that measurement of the concentration alone might not reflect the true changes in the circulating protein. Therefore, in this study, an attempt was made to determine the effect of ACTH on the regeneration of plasma protein by measuring the total circulating protein before and after depletion.

Admittedly, the limitations of this small series of experiments do not permit definite conclusions regarding the mode of action of adrenocorticotrophin in nitrogen metabolism. However, it was noted that following depletion the treated animals showed on the average a greater increase in the plasma volume, non-protein nitrogen, plasma protein concentration and total circulating protein than did the controls. The difference was not marked, as it

was 1.5 - 1.6% in the plasma protein concentration and approximately 5 - 9% in the other measurements.

It is interesting to note that the mean increase in recovery of the plasma protein concentration in the treated animal was considerably less than the increase in recovery of the total circulating protein. This is in keeping with previous observations that alterations in the plasma protein are not as apparent in the concentration as in the absolute measurements (181, 183, 184).

The technical difficulties encountered in this method of study and the paucity of the series of experiments have undoubtedly contributed to the inconclusiveness of these results. Other factors, which must be considered, are the dose of ACTH and the interval allowed for its action. The 5½ hour period was arbitrarily selected, since it was shown by Cutting and Cutter (67) that after a similar amount of depletion, complete regeneration occurred in 12 hours in untreated animals. It was felt that the 5½ hour period might be insufficiently long to allow the ACTH to exert its effect on protein metabolism, since Sayers, White and Long (228) produced marked depletion of the lymphoid tissue in mice in 3 - 6 hours after the administration of 1 mgm. of ACTH. Nevertheless, it is possible that sufficient time was not allowed for the maximum effect of the hormone. Engel obtained a satisfactory response with a dose of 2 mgms. of ACTH in his studies on urea formation in nephrectomized rats; but he allowed a longer period of time for its action (304). In the interpretation of these results,

it must also be remembered that the animals were exposed to a considerable amount of stress which is accompanied by the endogenous secretion of adrenocorticotrophin (226). This would also tend to mask the effects of the exogenous dose of ACTH in the treated animals.

Nevertheless, the results of this small series of experiments show that following the administration of ACTH, there is on the average a greater increase in the plasma volume, plasma protein concentration, non-protein nitrogen and total circulating protein than in the control group, although this difference was not marked. The increase in the plasma protein, which is in keeping with the hypothesis of Browne (1), was more apparent in the measurements of the total circulating protein than in the concentration measurements. The concomitant increase in the non-protein nitrogen in the treated animals does not necessarily indicate a catabolic effect on the protein molecule. The non-protein nitrogen is a more labile fraction, especially in the presence of acute hemorrhage where there may be a variable impairment of kidney function. Moreover, in accordance with the concept of a dynamic state of protein metabolism (2, 149), it is conceivable that with the increase in circulating protein there is a secondary catabolism of protein, in order to maintain homeostasis.

In conclusion, it may be said that the results obtained from these experiments offer nothing more than suggestive evidence that adrenocorticotrophin may cause an increase in the circulating

protein, which is more apparent in the measurements of the total circulating protein than in its concentration.

SUMMARY

(1) The literature concerning the factors affecting plasma protein formation has been reviewed. In this connection, special attention has been paid to general protein metabolism, the effects of pituitary and adrenal hormones and the relationship of plasma protein concentration to plasma volume.

(11) The washing-out and perfusion technique for the measurement of plasma volume has been described together with its limitations.

(111) The effect of ACTH on the plasma protein concentration, N.P.N., plasma volume and total circulating plasma protein was determined following massive hemorrhage in the rat.

(1V) A small mean percentage increase in all the measurements was found in the treated animals as compared with the controls.

(V) The increase in plasma protein was more apparent in the measurements of the total circulating protein than in the concentration, due to the masking effect of the increased volume.

(V1) The results of this small series of experiments merely offer suggestive evidence that ACTH may cause an increase in the plasma protein. This is in accord with the hypothesis that the hormone may exert its effect on nitrogen metabolism by mobilizing protein from the tissue for use in other parts of the body in times of emergency.

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