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STUDY OF SERUM PROTEIN PATTERN IN CANINES IN HEALTH AND IN CASES OF ASCITES

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INTRODUCTION

Hypoproteinemia is a condition characterised clinically by loss of weight, lassitude, unthriftiness and progressive development of edema in dependant parts of the body and by marked reduction of various components of plasma proteins. Usually it is the albumin fraction that is depressed.

The main factors responsible for hypoproteinemic state in animals are (a) excessive loss of proteins from blood (b) inadequate supply of proteins (c) defective absorption of protein (d) inadequate utilization of proteins and (e) impaired synthesis of plasma proteins due to diseases of liver.

In chronically ill patients, there is inanition, loss of weight, debility, low albumin level in blood and nitrogen loss. In nephrosis there is proteinuria, resulting in hypo-proteinemia. In cirrhosis of liver there is failure in plasma protein synthesis due to damage of liver parenchyma. Extensive burns and haemorrhage cause depletion in circulating protein particularly albumin and also fall in total plasma protein levels. Deficiency of protein occurs also in digestive disorders when lack of appetite caused inadequate intake, absorption and utilization. In severe trauma following major surgical procedures hypoproteinemia occurs. Hypoproteinemia also occurs in malignancy, untreated diabetes mellitus, hyper-thyroidism, haemorrhage and chronic systemic diseases. Undernutrition from moderate to severe degree are commonly seen in dogs as the result of neglect and ignorance.

The normal functions of plasma proteins are (a) maintenance and stabilization of blood volume (b) regulation of equilibrium levels of fluid exchange between intravascular and extra vascular components (c) transporting agent for the various nutrients and metabolites and (d) supplying the protein need of the tissues.

The normal values of plasma protein in health in dog, reported by different authors by electrophoretic technique are given in table II.

Hypoproteinemia brings about a fall in the osmotic pressure in the capillary bed and the fluid escapes into the tissue spaces resulting in edema. The circulatory efficiency is impaired and a tendency for venous stasis occurs in the lungs, liver and splanchnic lake. Strain on the heart brings about an impaired output. Gastrointestinal stasis occurs due to hypomotility. Hypoproteinemia interferes with fibroblastic repair that usually precedes callus formation in injury to the bone. Consequently healing of wounds and fractures is delayed. Hypocalcaemia occurs in hypoproteinemia, as 50% of serum calcium in health is bound to serum proteins.

Ascites is accumulation of fluid, non-inflammatory in character in the peritoneal cavity. It may result from conditions where the heart's action is impaired due to disease or due to pressure on the portal circuit occuring in cirrhosis of the liver or due to disturbance in osmotic equilibrium at the capillary end occuring in hypoproteinemia, anaemia and severe parasitism or due to faulty salt and water metabolism occuring in diseases of adrenals and kidneys.

The same factors which determine the edema formation also influence ascites formation. They are (a) pressure within the capillaries forcing fluid out of the vessels (b) osmotic pressure of plasma proteins drawing the fluid back to the vessels and (c) degree of capillary permeability. Any factor disturbing this normal equilibrium will permit the flow of fluid into tissue spaces with the production of edema. If the fluid accumulates into the peritoneal cavity ascites results. In cirrhosis of the liver there is portal congestion and because of the loss of functional tissue, synthesis of plasma protein is impaired resulting in hypoproteinemia and further transudation into the abdominal cavity.

The recognisable symptom in ascites is distension of the abdomen. The abdomen assumes a characteristic pear shape and by percussion a characteristic quiver can be felt on the opposite side. There is loss of weight, with prominent ribs and an increase in the hollow of the flank. Dyspnoea is present, the severity depending upon the amount of accumulated fluid in the peritoneal cavity causing pressure on the diaphragm and lungs. The dog may become totally anorectic with frequent episodes of vomition. The temperature is elevated in the beginning and fluctuates within normal limits once the ascites has occured.

In normal state of health, plasma proteins occur in a definite proportion. Pathological conditions bring about both quantitative and qualitative changes in the plasma proteins. Hence the study of plasma proteins in disease has yielded interesting and useful data.

Early precipitation techniques of crude separation of plasma proteins has revealed either hypoalbuminemia or hyperglobulinemia in disease. Where the dam suffered from hypoproteinemia the offsprings have been found to be unthrifty and weak. In diseases like multiple myeloma, cirrhosis liver, Kala-azar and lymphogranuloma venereum, the plasma protein pattern is quite diagnostic. In pregnancy, the total serum protein level will be reduced and this is just a reduction of the albumin level. The globulin will be changed only very little or not at all. This is in contrast to what happens with endometritis or pyometra. In such cases there will be instead, a marked rise in globulin. In hepatic cirrhosis there is protein leak into the body cavities. In such cases repeated removal of ascitic fluid depletes the system of essential proteins (albumin). The reduction in albumin may be primary or secondary due to rise in globulin. Rise in alpha-globulin occurs in conditions where there is challange to protein homeostatic mechanism as in acute febrile disease, inflammation, tissue destruction, nephrosis, and cirrhosis. In cirrhosis and ascites as the disease progresses the serum albumin falls and globulin rises reflecting the inability of liver to manufacture enough protein. Rise in beta-globulin denotes inefficiency in lipid transport. Rise in gamma globulin occurs in response to the foreign or endogenous irritants. (chronic infections) · In conditions where low levels of gamma globulins have been observed, a poor prognosis is indicated.

During the course of treatment periodic examination of serum protein pattern may be of prognostic value since a shift from dysproteinemia to euproteinemia would indicate improvement in general health. The study of plasma protein provides evidence in estimating the severity of the disease and is utilized as guides in treatment notably in cirrhosis liver, nephrosis, kala-azar, lymphogranuloma venereum and marked malnutrition states and haemoconcentration. Before distemper immunization as a routine the state of plasma protein level is assessed. With low plasma protein there is poor risk and breaks are likely to occur in immunization because antibody production is slowed down.

Ascites in canines is frequently encountered in the Madras Veterinary College Hospital. Table I shows the incidence of ascites in the Madras Veterinary College Hospital during the three years 1964, 1965 and 1966. The over all incidence is not very great. However cases of malnutrition in dogs being frequent, owners of dogs have to be educated about proper balanced feed for these animals, before an irreversible damage of hepatic cirrhosis with ascites occured.

The present study has been designed to assess the value of serum protein pattern in the diagnosis and prognosis in cases of ascites of hepatic origin in dogs. The incidence of this condition in the Madras Veterinary College Hospital is also studied. The electrophoretic technique for the separation of various factors of plasma protein and interpretation of the findings in the light of clinical picture have also been attempted.

REVIEW OF LITERATURE

Hypoproteinemia is characteristic of several diseased states wherein there is a marked decrease in plasma proteins. It indicates a low tissue protein content also. When stored fats and carbohydrates of the body are exhausted through conversion into calories, the protein reserves of the liver and other tissues are converted into calories for energy (36). Clinically it is characterised by loss of weight, excessive interstitial fluid, tendency to tire easily, low urine output, abdominal distension, increased pulse rate, fall of blood pressure, general malaise and debility (59). Various degrees of undernutrition from moderate to severe are commonly seen in dogs as a result of neglect and ignorance. A number of diseases result in deficiency of proteins and calories due to inadequate intake, absorption and utilization. In man injuries as fracture of long bones, trauma, burns and major surgery and many infectious diseases will deplete the protein store (32).

Decreased total protein levels are principally due to a reduction in albumin fraction. This occurs in several conditions as malnutrition, chronic systemic diseases, malingnancy, some liver diseases, last stages of pyometra, ascites of hepatic origin, repeated removal of ascitic fluid, recurrent or prolonged cardiac decompensation and gastrointestinal diseases with prolonged vomition and diarrhoea (12).

Excessive destruction of proteins, inadequate intake and absorption will cause hypoproteinemia as hyperthyroidism, high fever, major operations, excessive tissue trauma, severe

infection and ulcerative or infectious diseases of gastro intestinal tract (59). Hypoproteinemia occurs in conditions of continuous haemorrhage, parasitic infestation, renal disease, liver damage, hepatic fibrosis, congestive heart failure, anasarca and prolonged draught (9).

Rats experimentally fed with protein free diet showed signs of hypoproteinemia. The albumin fraction was sensitive to protein free diet (55). Dogs kept on low protein diet for ten weeks showed reduction in albumin to one half of the normal value. The loss in total circulating plasma albumin represented three percent of total body protein loss. In similar experiments dogs were fed on diets containing 1.6 G. of protein per day for 13 weeks. The albumin was reduced to one half of the normal value and a recovery on a high protein diet was extremely slow. After eleven weeks the levels were only 80% normal. It was reported that for every 30 G. of tissue protein lost or gained 1 G. of total circulating protein was lost or gained. The total protein study in concentration camp victims cared for in Swiss Hospital during the World War II revealed the following data:

(a) Patients with no edema the total protein was 5.8 G%

(b) Patients with slight edema had 5.0 G%

(c) Patients with massive edema showed 4.7 G%

(d) Patients with severeedema showed 4.0 G%

The Albumin was reduced to one half and the alpha-2 and beta globulins were normal or slightly more (47).

In starvation the body protein is degraded to amino acids, deaminated and oxidised. This is an obligatory loss and cellular

functions deteriorate precipitously (27).

The effect of protein depletion on distribution of protein synthesis in the dog was studied by Garrow (24). It was observed that after 50 to 60 days on protein free diet the weight had fallen down to 13 to 18%. In later stages edema was detected. There was a rapid reduction in the amount of circulating proteins.

Hypoproteinemia is present in chronic liver diseases. There is reduction in albumin and albumin globulin ratio is reversed (39).

The effects of inanition, acute and chronic protein depletion and repletion with several levels of proteins, on the distribution of serum proteins in adult rats were investigated by filter paper eletrophoresis by Weimer (56). After protein depletion there were decreases in albumin, alpha - 1, alpha - 2 and beta globulin fractions. Purified diet containing 17% or more of casein not only restored all levels to normal values but increased total proteins.

PLASMA PROTEIN - NORMAL PATTERN - ROLE IN HEALTH

In normal healthy animals the serum protein contains four distinct fractions and they are (a) albumin (b) alpha globulin (c) beta globulin and (d) gamma globulins and plasma contains in addition fibrinogen as determined by paper electrophoretic technique (54). The normal plasma and serum protein patterns in dog have been studied by several authors. Deutch and Goodloe (21) and Levis, Page and Gasser (38) have determined the normal values for dog plasma using the classical moving boundary

electrophoresis. They have reported that good resolution between beta and gamma globulin did not occur in dog plasma. Zeldiss and Alling (60) have reported on the total plasma proteins and the amount of individual fractions. The results have been expressed in terms of absolute amounts and not in percentage. Moore (43) studying the serum protein pattern in 17 normal dogs using phosphate buffer at pH 7.4 has designated the various fractions by numbers from one to six in their decreasing order of mobility. Stockl and Boguth quoted by Vaselinovitch (54) Groulade (26) deWael (22) and Campbell (15) have determined the normal serum protein pattern in dogs using paper electrophoresis. Hoe (31) studied the serum protein pattern in 26 young and healthy dogs living under similar conditions by the same method and also studied the pattern in 140 dogs suffering from various diseases. Parthasarathy (45) studied the plasma protein pattern in normal dogs and dogs with eczema.

The normal values of plasma proteins in dogs in health as reported by different authors by paper electrophoretic technique are given in Table II.

The albumin fraction in the plasma protein is in excess of globulin in man, sheep, goat, rabbit, dog, rat, and guinea pigs. The albumin is equal or globulin predominates in horse, pig and cow. Although the normal values given for the dog indicate preponderance of albumin, it is not uncommon to observe in apparently normal animals that albumin and globulin occur in

equal amounts or globulin is in excess (12).

A detailed review about the functions of plasma proteins in health is given by Esdall (2). They are (a) maintenance and stabilisation of blood volume (b) transporting function of various products including enzymes and hormones (c) transport and mobilisation of antibodies (d) protection against blood loss by clotting mechanism. (Fibrinogen) (e) nutritive function and (f) transport of lipids. Other than buffer and transporting functions, the albumin provides mobile reserve source for amino acids and plays an important role in the regulation of plasma volume and tissue fluid balance. The albumin component is responsible for about 75% of total colloid osmotic pressure of the plasma. This high efficiency of albumin is due to its low molecular weight than the average values for plasma proteins and high net electric charge under physiological conditions (46). The following functions are also attributable to plasma proteins (a) viscosity - a factor responsible for the normal maintenance of blood pressure and (b) stability of blood. The globulin and fibrinogen fractions influence the tendency of the corpuscles to adhere to one another and form rouleaux or clumps (c) immune bodies associated with gamma globulins (7). The globulins perform a number of enzymatic activity in the plasma (27). The principal fraction of serum lipoprotein in the dog serum lies as alpha -1 lipoprotein in contrast to human serum lipoprotein which lies in beta globulin. The alpha lipoprotein in dogs contains about 70% total serum lipids (50).

PHYSIOLOGICAL VARIATION

A physiological variation in the plasma protein has been observed in pregnancy. At this time the total serum protein level is reduced and this is just a reduction in albumin level. Globulins will be changed only very little or not at all. In women the concentration of albumin is found to be reduced until term but does not return to normal level until a number of weeks after delivery (47). In ewes a reduction in albumin concentration has been reported during the first half of pregnancy but this returned to normal at term. The globulin in the serum of women increases and has been found to be greatest at term and for a period after delivery, whereas in ewes during the latter half of pregnancy a marked reduction in globulin has been observed. A reduction in the total protein in serum has been reported both in human and ewes throughout the gestation period (19).

Levis (37) conducted a study on the development of plasma proteins in dogs from weaning to adult age. The concentration of total protein and gamma globulin increased during the most rapid period of growth. During the first month the concentration of albumin was low but returned to initial level at six months of age and thereafter increased.

SYNTHESIS OF PLASMA PROTEIN

The hypoproteinemia seen in cirrhosis of liver provides clinical evidence of the importance of liver in the formation of plasma proteins, mostly albumin.

In experiments in which liver was poisoned with agents as chloroform and phosphorous, the blood fibrinogen fell rapidly in proportion to liver injury. Upon regeneration and repair of liver the plasma fibrinogen returned to normal. Miller et al (42) in their studies using C^{14} labelled lysine, perfused rat livers and demonstrated that the liver was capable of synthesising albumin, alpha and beta globulins and fibrinogen. The gamma globulin was synthesised extra hepatically by the reticulo-endothelial system. Ortega and Mellors (44) demonstrated that gamma globulin can be synthesised in vitro by lymphatic tissues. Lane, quoted by Cornelius (18) observed that plasma cells could synthesise gamma globulin as evidenced by the presence of large amounts of gamma globulin in mandibular palsmocytoma.

PLASMA PROTEIN IN DISEASE

In chronic protein depletion hypoalbuminemia with increased or normal globulin content in the plasma protein, occurs. A reduction in the actions of various enzymes of the gastro-intestinal tract has also been observed. A disturbance in the hormonal equilibrium and calcium phosphorous ratio results (18). The failure of liver to anabolise amino acids and protein during hepatic insufficiency is manifested by tissue wasting and fall in plasma protein. This may be sufficiently severe to cause edema because of lowered osmotic pressure of the plasma. The effect of disease upon transporting function of plasma protein (albumin) has been reviewed by Foster quoted

by Putnam (46). Marked lowering of plasma albumin is accompanied by a decline in total calcium. Delay in fibroblastic proliferation and subsequent delay in wound healing has been observed in hypoproteinemic dogs. Visceral edema due to hypoproteinemia causes pressure on the heart, lungs, liver and gastro intestinal tract. Hypomotility and atony of the gastrointestinal tract result. Impaired gastrointestinal functions and inadequate diet predispose to deficiency syndromes (58).

The first acceptable data in Lannec's cirrhosis indicating marked changes in plasma proteins was reported by Abrami and associates quoted by Anson (3) indicating hyphoalbuminemia and hyperglobulinemia. Luetscher quoted by Anson (5) recorded the first result of electrophoretic analysis of plasma proteins in cirrhosis and found that there was a reduction in albumin and approximately two to three fold increase in globulin. Ascitic fluid was found to contain 1/10th or less protein content than plasma, with all components represented in much the same distribution as in plasma except for relatively greater albumin peaks in some instances. Gray & Baron, Kabot and associates and Thorn and associates as quoted by Anson (5) have made detailed analysis of plasma protein in Cirrhosis and have found hyphoalbuminemia and hyperglobulinemia in such cases. The mean serum albumin level in 28 cases of cirrhosis without ascites has been found to be 3.7 G% while 43 cases of cirrhosis with ascites showed values of 2.3 G%. This indicates a greater drain of plasma albumin in cases of cirrhosis with ascites (6).

Marrack and Hoch (40) state that in diseases involving liver parenchyma, the serum protein is characterised by an increase in gamma globulin and decrease in albumin, some times to very low levels. Zollner and associates quoted by Polson (49) have observed an increase in beta globulin as a most constant finding liver damage. They have also referred to the drop in albumin as evidence of acute liver damage.

Groulade (26) in his study on 43 diseased dogs observed that in febrile condition the albumin was reduced and alpha -1 and alpha -2 globulin were elevated. In chronic nephritis the alpha -2 component was raised. In general in liver disease the albumin was lowered and globulin was elevated.

The first extensive study on serum protein by filter paper electrophoresis in domestic animals was done by Boguth quoted by Vaselinovitch (54). The following findings were observed: (a) alpha globulin fraction was elevated in acute inflammation in dogs and swines. (b) Alpha and gamma globulin elevations were observed in sub-acute inflammation as polyarthritis, various infectious diseases and chronic nephritis in dogs. (c) Gamma globulin was elevated in chronic inflammation and cirrhosis liver. (d) Beta and gamma globulin elevations were seen in hepatitis and other liver damages in dogs and cattle and hyperplastic endometritis and pyometra in dogs. (e) Alpha beta and gamma globulin were elevated in malignant disease.

Polson (49) studied the changes in electrophoretic pattern in sera of dogs suffering from various canine diseases-babesiosis,

rickettsiosis and distemper. The results obtained indicated the diseased state but were not diagnostic. Mogle and associates quoted by Bloom (12) studied the serum protein pattern on renal disorders in 63 dogs. The nephrotic syndrome was characterised by hypoproteinemia with a fall in albumin and gamma globulin with marked albuminuria and tendency towards ascites, but without a rise in nonprotein nitrogen. Dewael (22) in his study on serum protein in canines by paper electrophoretic technique states that in all pathological cases the mean albumin percentage was lowered significantly. The alpha -1 globulin was particularly unaffected by the diseases examined. There was significant relative increase in alpha -2 globulin in hepatitis and tumours. In malignancy the beta globulin was raised significantly and in benign and malignant tumours gamma globulins. Campbell (15) observed that in hepatitis there was marked rise in gamma globulin and reduction in albumin. In dogs in acute infection, increase in alpha globulin was evident. In distemper there was an increase in gamma globulin content. In pyometra the beta globulin fraction was elevated and in peritonitis the alpha globulin fraction was increased. In his review on analysis of serum proteins, Vaselinovitch (54) observed (a) important and dynamic changes in serum protein patterns in dogs and pigs. (b) Primary diffused damage of liver usually resulted in hypoproteinemia due to low production of albumin by liver. Changes taking place in globulin fraction might be secondary in nature. (c) Serum

protein in nephrotic syndrome appeared to be pathognomonic and (d) any stimulation of reticuloendothelial system such as infection or active immunisation resulted in primary hyperglobulinemia. Parthasarathy (45) studied the serum protein pattern in normal dogs and in dogs affected with eczema by filter paper electrophoresis. The albumin was reduced and beta globulin was increased in 60% of cases. There was elevation in gamma globulin. The total protein and alpha globulin were not elevated or altered much.

PATHOGENESIS OF ASCITES

Ascites is a collection of fluid, non-inflammatory in nature in the peritoneal cavity and is essentially a symptom than disease (33). It may result from general causes which involve heart, liver, and kidney. It is seen in association with anaemia and severe parasitism disturbing the osmotic equilibrium of the circulating blood. The portal circulation and liver are mutually interdependent, the liver depending upon the portal vein for its supply of nutrients and the portal flow depending upon the patency of hepatic sinusoids. The passage from portal circuit through the liver to the caudal venacava is dependent upon the patency of hepatic vascular bed and obstruction results in damming back of blood in the portal system, interference with digestion and absorption and in final stages in the development of ascites (9). There is failure to anabolise the amino acids and proteins during hepatic insfficiency manifested by tissue wasting and fall in plasma protein. This

may be sufficient to cause edema because of lowered osmotic pressure of the plasma. If there is obstruction to portal circulation as in hepatic fibrosis edema is much more severe and ascites results (10). In cirrhosis liver there is increased capillary permeability with loss of colloidal proteins into tissue with edema or ascites or both (34). Low albumin level and increased portal vein pressure are the major contributing factors for the production of ascites. A new steady state is set up in which body water is increased, sodium levels are lowered, volume of urine becomes small and salt excretion is limited (23). Himsworth (29) is of opinion that the same factors which determine edema formation also influenced ascites formation. Hypoproteinemia contributes to the production of ascites in liver cirrhosis. In cases of ascites accompanied by nephrotic syndrome there is always generalised edema.

Boltun (13) experimentally produced pathological changes in the liver resulting from passive venous congestion. He partially obstructed the inferior vena cava above the liver, thereby producing portal hypertension. Within 24 hours, ascites developed which however gradually disappeared after two or three months, when a collateral venous circulation developed. Ralli quoted by Himsworth (30) found that urine in cases of liver disease with ascites contained considerable amount of an anti-diuretic principle resembling pitressin. It was observed that the accumulation of fluid was seen in the peritoneal cavity without gross generalised edema in ascites of liver disease.

This localisation was attributed to (a) portal hypertension (b) local increase in capilliary permeability and (c) defective drainage by abdominal lymphatics (30). The presence of salt retaining hormones in ascites of liver origin has been attributed to (a) the inability of the liver to inactivate the hormone and (b) the adrenal cortical hyperfunction. The adrenal is concerned in sodium retention in the kidney tubules and is brought about by a group of adrenal steroids and antidiuretic hormone of posterior pituitary (16). Gordon (25) states that some adrenal steroids do increase the sodium reabsorption from kidney tubules. Bongiovanni quoted by Child (17) observed high corticoid levels in urine in cirrhotic patients.

Electrolyte shifts seen in cirrhosis seem to be governed largely by adrenal mineralocorticoids and possibly by aldosterone (14). The walls of lymphatic vessels are attached to fibers of intercellular substance. These fibers are put on stretch as the tissue becomes spread apart by fluid. They pull on the walls of lymphatic vessels in various directions and hold them open. Thus the lymphatic vessels do not collapse in edema (28). A continuous circulation of protein from blood and extra cellular fluid into lymphatic system and back again into blood has been reported. The rate of transfer of labelled albumin across the peritoneal membrane was found to be three times faster than that of globulin in terms of weight. The albumin fraction also reached an equilibrium much more rapidly between blood plasma and ascitic fluid than globulin (18).

The different methods of serum protein assay depend upon their precipitation characters in salts like ammonium sulphate and sodium sulphate or water miscible organic precipitants at low ionic strength and low temperature as ethanol or by electrophoresis based upon the principle of migration of charged particles of protein in an electrical field and by ultracentrifugal analysis and immunological techniques. Assay is also done by determining the total protein content by Kjeldahl method, biuret method and refractometric methods (3).

SERUM PROTEIN PATTERN-VALUE IN DIAGNOSIS AND PROGNOSIS IN LIVER DISORDERS.

Several authors (4), (5), (40), (49) and (54) have observed a low albumin and high globulin in cirrhosis of the liver.

Franklin et al quoted by Child (16) while studying the electrophoretic pattern in liver diseases observed that progressive change towards normal in electrophoretic pattern coincided with clinical improvement of the patient studied.

Periodic examination of serum protein during clinical observation and treatment may be of prognostic value. A slight shift from dysproteinemia to euproteinemia would indicate improvement in general health of the animal (54). Putnam (48), in his review on plasma protein in disease has summarised the following observations. (a) The reduction in albumin may be primary or secondary due to rise in globulin and altered colloid osmotic pressure. (b) Rise in alpha globulin occurs where there is challange to protein homeostatic mechanism

as in acute febrile disease, inflammation, tissue destruction, nephrosis and cirrhosis.(c) Rise in beta globulin denotes inefficiency of lipid transport and (d) rise in gamma globulin occurs in response to foreign or endogenous irritants. Dewael (22) states that on the basis of changes observed in serum protein pattern, differential dignosis can be made. Even storing the serum at - 20°C has not altered the results. Paper electrophoresis has proved very useful in cases of ascites. If the strong beta and gamma globulin bands characteristic for liver cirrhosis are absent we may exclude this disease as a cause for ascites. Campbell (15) is of the opinion that filter paper electrophoresis may be of great value to the clinician in assessing the severity of a condition and in following the recovery or progress of an illness by observing the changes in plasma protein. However from a comprehensive review on the results of sera in different diseases Marrack and Hoch (40) have concluded that, the changes in electrophoretic pattern cannot be regarded as characteristic or specific for any particular disease. They regard these changes in the same light as determination of erythrocyte sedimentation rate in that they are a measure in the clinical state of the patient rather than specific evidence of disease. Sunderman and Sunderman (52) have made detailed review and studies on clinical application of fractionation of serum protein by filter paper electrophoresis and on the causes of discrepancies in fractionation of serum proteins by the said method.

PLASMA PROTEINS - THERAPEUTIC USES

Lyophylised canine plasma was successfully used in dogs for the treatment of shock resulting from haemorrhage, accidents or surgery. In a study of 47 dogs in shock, infusion of 50 ml. of reconstituted plasma at the rate of 50 drops per minute was usually effective. Infusions were repeated after one hour in some cases (51). The blood plasma transfusion have been fairly extensively used in malnourished patients in an attempt to correct protein deficiencies. Study of nutritional values of plasma protein when given by mouth has resulted in a variety of results. Following oral administration with precipitated washed bovine serum, a positive balance was achieved in dogs. But injected serum protein was definitely superior in correcting the conditions. Protein by vein was a little more completely utilized to form new protein in the body than the same protein given by mouth.(1). Thron et al quoted by Child (16) reported good results in the use of human albumin for treatment of hypoproteinemia.

Therapeutic potentials of highly purified normal human plasma albumin and gamma globulin and protein hydrolysates are being actively investigated and have found important applications. The results of serum protein infusions are not always successful because of persistance of those factors leading to accumulation of fluid in the peritoneal cavity, with which increase in serum albumin is apparently unable to cope up.

From the review of literature, it is observed that hypoproteinemia in varying degrees occurs in undernourished dogs as a result of neglect and ignorance, that it is an accompaniment in a variety of disease conditions, that liver is the principal organ for the synthesis of plasma protein, that organic and functional disorders of the liver interfere with this synthesis, that in well established hypoproteinemic states recovery on high protein diet has been found to be extremely slow, that cellular functions deteriorate precipitously in starvation because of the break down of endogenous proteins, that in health wide variation exists in the distribution of various components of plasma protein among the different species of animals that albumin fraction in serum provides a mobile reserve source for amino acids and plays an important role in the regulation of plasma volume and tissue fluid balance, that in hypoproteinemia the decreased total protein levels are principally due to a reduction in albumin fraction that in cirrhosis of the liver electrophoretic analysis of plasma proteins has shown a reduction in albumin and apparently 2 to 3 fold increase in globulin, that in cases of cirrhosis with ascites a greater drain of plasma albumin has been noticed, that failure of the liver to synthesise plasma proteins and its inability to inactivate the salt retaining hormone are the two factors contributing to the occurence of edema and that in liver disease a progressive change towards normal in electrophoretic pattern coincided with the clinical improvement of the patient.

MATERIALS AND METHODS

Twelve adult nondescript dogs, five females and seven males free from ecto and endo parasites, apparently healthy and fair in condition, were selected at random from the lethal chamber of Madras Corporation as control for estimating the standards of blood serum proteins and haemogram.

From among the cases of ascites in dogs that attended the small animal clinic medical unit of the Madras Veterinary College Hospital, twelve cases were taken for this study. They comprised of six males and six females. Among these, six belonged to non-descript breed, four alsatian and two dachshund. Their weight ranged from ten pounds to sixty pounds and their age from ten months to eight and half years. Diagnosis of ascites was based on history of the case, distension of the abdomen and by the characteristic fluid thrill on percussion of the abdomen. In certain doubtful cases skiagrams were taken to rule out pregnancy or other abdominal tumours. They were subjected to a systematic and standardised clinical examination in the proforma given below. In addition, the study comprised of analysing the previous records of the college hospital corresponding to the years 1964 and 1965 with a view to assess the incidence of this condition among dogs.

PROFORMA FOR INVESTIGATION INTO THE CAUSES OF ASCITES IN DOGS

1. Case number

2. Description of the animal

   i. Age

   ii. Breed

   iii. Sex

3. Owner's name and address.

4. History and duration of illness

5. Condition of the animal

6. Weight at the time of admission

7. Measurement of the abdomen

8. Liver enlargement, if palpable even after tapping the fluid

9. Type of feed given

10. Whether any animal protein was given in the diet

11. Exercise and its nature

12. Nature of feed utilisation (Motion etc.)

13. Whether treated for any complaint for worm, indigestion, fever, etc. earlier in the veterinary hospital.

14. Haematology T.C., D.C., Hb., P.C.V., and E.S.R.

15. Urine analysis.

16. Microscopical examination of faecal samples

Electrophoretic studies of the protein pattern with serum samples from apparently healthy and those from cases of ascites were also undertaken.

i. Examination of the faecal sample was done as a routine for the presence of eggs of parasites.

ii. Total R.B.C. count: with a double NEUBAUER haemocytometer using Hayem's fluid as a diluent.

iii. Total W.B.C. count: with haemocytometer, Thoma's fluid was used as a diluent. (Care was taken to complete these procedures within three hours after collection of the sample).

iv. Erythrocyte sedimentation rate: with Wintrobe haematocrit tubes, the rate of sedimentation recorded after the end of one hour.

v. Haemoglobin estimation: by acid haematin method with Sahli's (Hellige type) haemometer.

vi. Packed cell volume: with Wintrobe haematocrit tubes after centrifuging at 3000 rpm. for half an hour.

vii. The differential leucocytic count: with Wright's stain by battlement method.

The results of haematological studies of the apparently healthy dogs and those from cases of ascites have been incorporated in the tables IV and V.

Collection of blood Serum:

About three millilitres of blood were collected from external saphenous vein of dog through a sharp dry sterile needle into a clean and dry sugar tube. It was kept without shaking for one hour and then centrifuged for half an hour at 3000 rpm. The clear serum was pipetted out in a small clean, dry penicillin vial, labelled and stored in the freezing chamber in the refrigerator.

Electrophoresis:

Electrophoresis was carried out by the horizontal open strip method, in a tank that was designed as per the description

of Grassmann and Hanning (8). The equipment was supplied by ADAIR DUTT and CO., MADRAS.

Preparation of Veronal buffer: 1.84 grams of diethyl barbituric acid and 10.3 grams of sodium barbiturate (Analar) were weighed in the mettler balance and dissolved in one litere of distilled water (53). The pH. of this buffer was 8.6 and of ionic strength 0.05.

Setting up of the appartus: The tanks were filled up to the mark with the buffer. Strips of Whatman No.1 filter paper were cut in sizes of 4 cm. into 34 cm. A linear pencil mark was made six centimetre away from one end. The label number of the specimen to be applied over the filter paper was also marked. The filter paper was soaked in the buffer solution and the excess of buffer was pressed out between the folds of a clean blotting paper. Then the strip was applied over the plastic frame, dipping the ends of filter paper in the buffer solutions. The pencil mark was near cathode. The tank was closed and a current applied for half an hour to obtain equilibrium. (300 volts). 0.02 ml. of serum sample was applied over the strip near the cathode by the side of the pencil mark, using a Sahli's haemoglobin pipette. A current with a constant potential gradient of 300 volts was applied for six hours. At the end of six hours the strip was taken out and dried in a hot air oven at 120°C for thirty minutes. The stain used was 0.1 per cent bromophenol blue in methanol. The strip was soaked in the stain solution for six minutes and

the excess of stain was washed in five per cent acetic acid solution for five minutes. The washing was repeated thrice with seperate solutions of acetic acid labelled one, two and three, till the back-ground was clear. The strip was dried and exposed to ammonia fumes when the seperated bands took intense blue colour.

Calibration:

It was done by using a densitometer over a graph paper. The total number of squares were counted and percentage of each fraction was calculated and the results tabulated.

The following points were observed as a routine:

1. The smooth side of the filter paper was used upwards.
2. The buffer solutions were always maintained equal in volume in the two compartments of the tank.
3. The current was switched off during the application of the serum.
4. The application of serum was made close to the negative electrode.(Cathode).
5. The buffer solution was changed once in eight runs.
6. The polarity was reversed at the begining of every experiment.
7. Excessive water vapour which got collected on the glass lid during the experiment was wiped out then and there.

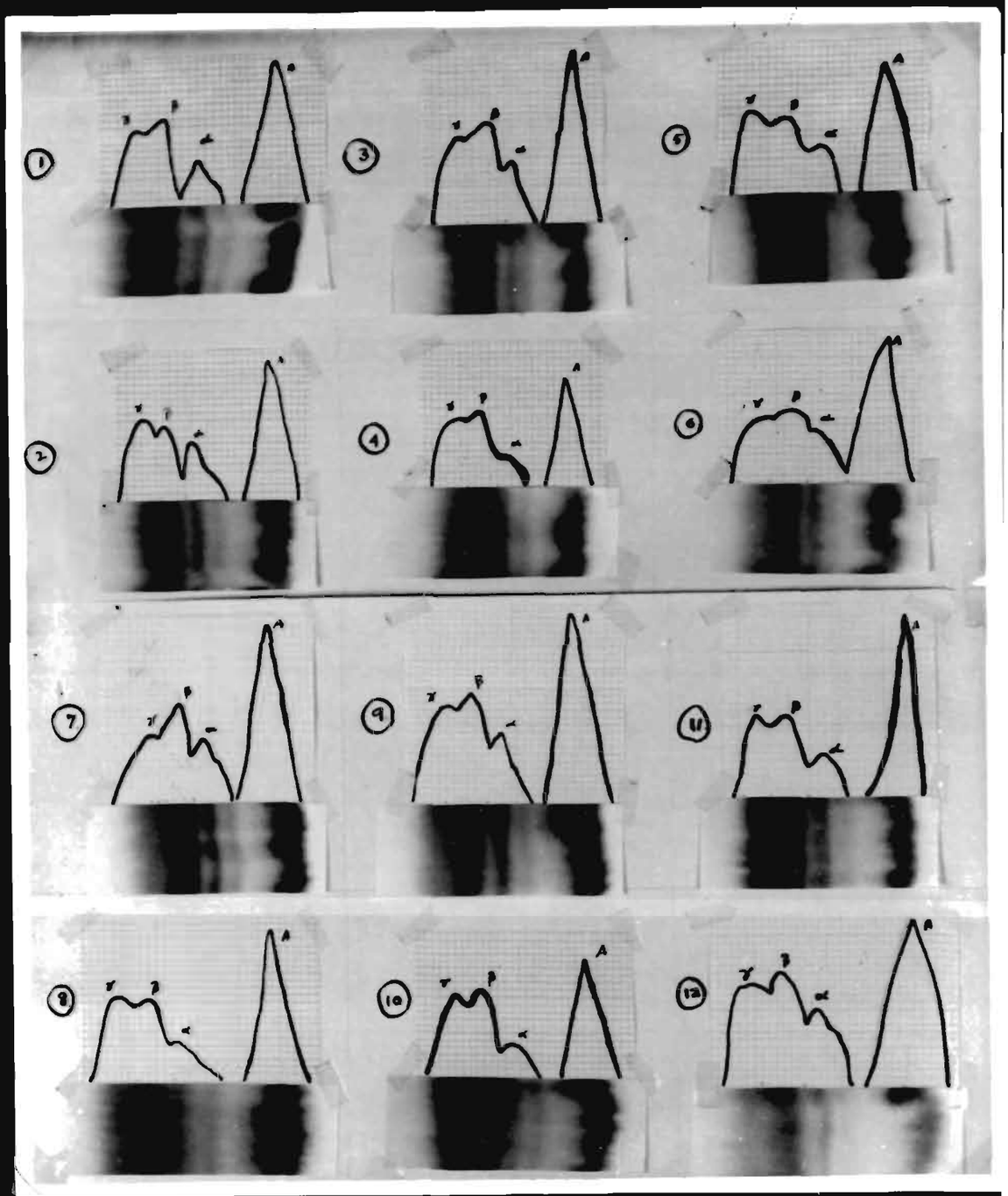

PLATE 1. PROTEINOGRAM - NORMAL DOGS.

RESULTS OF EXPERIMENTATION AND ANALYSIS OF THE DATA OBTAINED

The serum protein in twelve cases of ascites in dogs were studied and data collected were analysed. Among the cases of ascites, it was found that six cases were fed on vegetarian feed. These had no access to any animal protein. Others were fed with some animal protein mostly made up of fascia and bones. Some were fed with beef, mutton and eggs. No exercise was given to any animal. All cases had intestinal stasis. Three cases had history of earlier treatment for indigestion or parasitism in the college hospital. They were case No.5294, 11085 and 12805 of the out-patient unit, small animal clinic.

Dignosis of ascites was based upon history and symptoms noticed. In case No.6262 the liver was palpable, enlarged and soft. A skiagram revealed the enlarged liver shadow in this case. Exploratory laparotomy was done in this case and the liver was found enlarged with neoplasms. On post-mortem examination the tumor was diagnosed as cholangiolar adenoma. Case No.11437 was a well built, fair looking alsatian bitch. It had no previous history of mating. Skiagram did not reveal anything significant to be of diagnostic value. The characteristic thrill of the abdomen was observed only on very careful examination and there was no clear distension of the abdomen. The animal developed ascites during the course of treatment and the post-mortem examination revealed cirrhosis of the liver. The symptoms in the rest of the

cases of ascites were quite familiar and diagnostic.

Liver was palpable in four cases. They were, case No.6262, 6410, 11085 and 11786. Case No.6262 at autopsy showed malignant growth in the liver-cholangiolar adenoma. Edema in the dependent parts was present in three cases besides ascites. They were case No.10902, 11786, and 12581. History further showed that in two cases (i.e., 10902 and 11786) edema and ascites developed simultaneously. Case No.12581 developed edema of dependent parts after the development of the ascites, the animal was discontinued treatment and died later. Urinalysis and post-mortem could not be done in this.

Five dogs died during the course of treatment and the post-mortem examination conducted in four of them showed the following, pathological changes in the liver.

5294 .....Atrophic Cirrhosis -liver

6262 .....Cholangiolar adenoma -liver

11437 .....Cirrhosis -liver

11786 .....Cirrhosis -liver, and chronic nephritis.

Post-mortem examination could not be done in the case No.12581.

Haematology:--There was anaemia in six cases characterised by a reduction in both haemoglobin and red blood cells as compared with control dogs. In all cases except in case No.11437, there was leucocytosis with neutrophilia. However, there was a depression of neutrophils in case No.5294. This case showed a high eosinophilic count. This was suffering from hook-worm infection in addition. The erythrocyte sedimentation

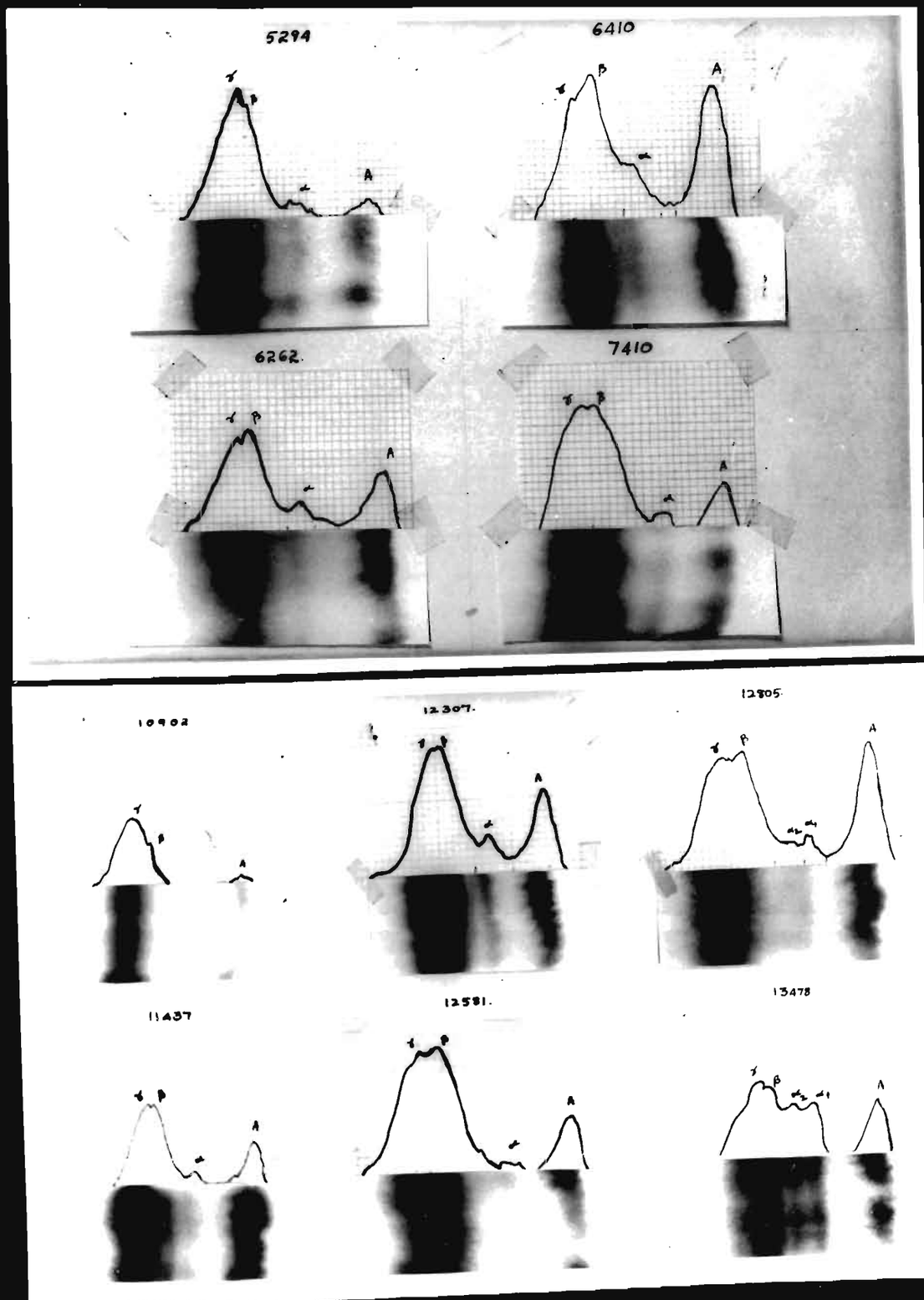

PLATE 2. PROTEINOGRAM - ASCITES IN CANINES.

rate was elevated in four cases. They were case No.6262, 11085, 11437 and 12805. Case No.6262 had malignancy liver and case No.11437 had cirrhosis of liver. The urinalysis could not be done in all animals. Faecal examination revealed eggs of ancylostome species in two dogs. They were case No.5294 and 7066. In others there were no eggs of parasites.

Electrophoresis: It was observed as a result of electrophoretic studies on all the sera collected from the cases of ascites in dogs, there was significant reduction in albumin fraction while percentage of globulin was elevated in all cases, particularly beta and gamma fractions. Alpha globulin fraction split was seen in four cases and in three of these, there was also an elevation of this fraction as compared to normal healthy proteinogram. Elevation of beta globulin was seen in eight cases. It was lowered in one case and normal in three other cases. Gamma globulin elevation was seen in ten cases. Two of these had more than sixty per cent.

Study of serum protein pattern during the progress of ascites was done in two cases. They were case No.11085 and 11786. They revealed progressive reduction in albumin and elevation in globulin. (Vide Table VIII)

The results of haematological examination and electrophoretic studies are appended in the Tables IV to VIII annexed.

DISCUSSION

Among the various functions of plasma proteins in health their role in the maintenance and stabilisation of blood volume and in the regulation of fluid exchange between intravascular and extravascular compartments has been extensively studied by various workers. The liver as the seat of plasma protein synthesis is well documented. Deficiency of protein may result in excessive loss of protein from the blood occuring in massive haemorrhage, inadequate supply of proteins, and impaired synthesis of plasma proteins occuring in organic or functional diseases of the liver. In such a situation a chain reaction is set up by which the cardiovascular and renal functions are impaired bringing about a circulatory stand still, disturbances in water and electrolyte metabolism and also mineral metabolism.

Cases of malnutrition in dogs are frequent. This may be the result of neglect and ignorance. Owners of dogs have to be educated about adequate and balanced feed for these animals. Otherwise a progressive hepatic damage would occur. In chronic cases when fibrosis of the liver has occured the liver is unable to synthesise plasma proteins and inactivate the salt retaining hormone and anti-diuretic hormone of the pituitary. This results in the transudation of fluid into the peritoneal cavity. It has been our experience that once the transudation occured the condition is irreversible.

In the cases of ascites studied three cases showed advanced

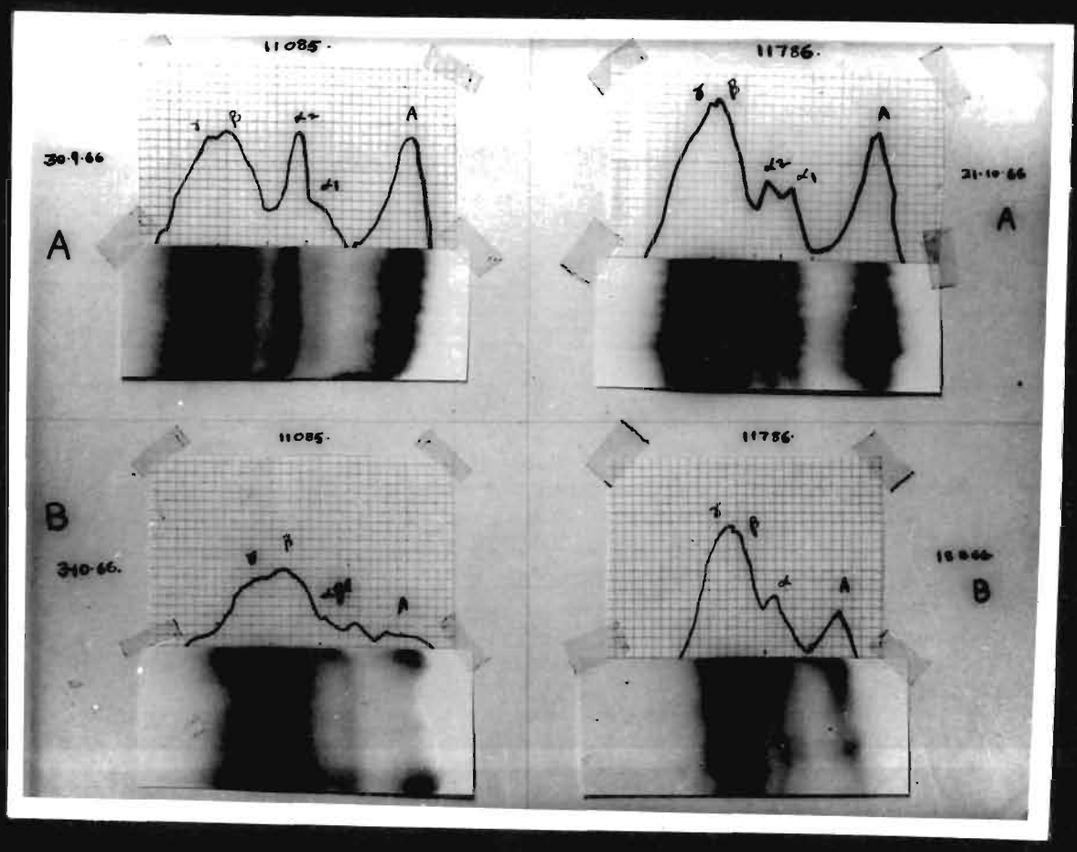

PLATE 3. PROGRESSIVE CHANGES IN PROTEINOGRAM IN ASCITES IN DOGS DURING THE COURSE OF THE DISEASE.

hepatic fibrosis on post-mortem. In these one had in addition lesions of chronic nephritis. Whether the liver damage occured earlier to the involvement of the kidney or not, could not be ascertained. However in both the conditions ascites occured. In advanced stage of liver disease symptoms attributable to diseased state in remote organs like kidney complicated the clinical picture. Case No.6262 showed at autopsy malignant neoplasm in the liver. The course in 7 cases could not be followed because they discontinued treatment at varying intervals. However all showed ascites and in two of them (6410, and 11085) the liver was palpable in addition.

Electrophoretic studies revealed in all cases of ascites a significant reduction in albumin while the percentage of globulins particularly the beta and gamma fractions was elevated. It was also found in the two cases where the electrophoretic studies of the serum protein were conducted during the course of the disease, there was a further reduction of albumin fraction to about a third in case No.11085 and a fall to about one half in 11786.

The anaemia and neutrophilic leucocytosis could be explained by the lesions in the liver. Eosinophilia in case No.5294 was due to the concomitant hook-worm infestation. The very low percent of albumin observed in this case is due to the failure of the liver to synthesise plasma proteins on the one hand and the depletion of blood by the parasites on the other in the intestines.

SUMMARY

1. The serum protein pattern in 12 cases of ascites in dogs was studied.

2. In all the cases there was a decrease in the albumin fraction and an increase in the globulin fraction.

3. There was varying degree of anaemia in all the cases and a neutrophilic leucocytosis.

4. The proteinogram in cases of ascites ranged from 4.17% to 33.49% in the case of albumin, from 0% to 25.25% in alpha globulin, from 16.68% to 36.45% in case of beta globulin and 18.32% to 79.15% in case of gamma globulin.

5. In the 12 apparently healthy dogs electrophoretic studies showed a range in the case of albumin from 35.29% to 48.96%, 8.9% to 14.28%, with alpha, 17.03% to 25.01% with beta and 18.06% to 30.78% with gamma globulin fractions.

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TABLE - I

INCIDENCE OF ASCITES IN CANINES IN THE MADRAS VETERINARY COLLEGE HOSPITAL DURING THE YEARS 1964, 1965 and 1966.

	1964	1965	1966
No. of cases attended	4551	5936	5399
No. of clinical cases of ascites	3	18	12
Percentage of incidence of ascites	0.07	0.30	0.22

TABLE -II

NORMAL SERUM PROTEIN VALUES OBTAINED BY DIFFERENT WORKERS

NAME OF WORKERS	No. of animals (Dogs)	Relative percentage of				REMARKS
		Albumin	GLOBULINS			
			Alpha	Beta	Gamma	
Moore (1945)	17	50 ± 4	26±5	10±7	14±3	Classical Electrophoresis Phosphate buffer P.H: 7.4
Stockl and Boguth(1953)	--	50.5	13.8	20.4	12.3	-
Groulade (1953)	15	60.5±3	11.5±2	20±2.5	7.6±1.5	Paper Electro-phoresis Veronal Buffer PH:8.6
Dewael (1956)	16	48.7	13.5	24.7	12.7	-do-
Campbell (1957)	17	47±5.15	12±1.58	14±2.73	25±4.11	-do-
Parthasarathy(1962)	10	44.49	9.56	18.42	27.53	-do-

TABLE - III

MEAN RELATIVE PERCENTAGE OF SERUM PROTEIN OF NORMAL AND DISEASED DOGS (DEWAEL - 1956)

Disease	No.	A †	GLOBULINS				
			Alpha-1	Alpha-2	Beta-1	Beta-2	Gamma
Normal	16	48.7	6.6	6.9	9.9	14.8	12.7
Hepatitis	10	35.1	6.6	15.0	B_1+B_2 = 27.2		16.2
Liver Cirrhosis	32	25.6	6.4	8.1	$B_1+B_2+\gamma$ = 59.90		
Benign Tumors	30	41.2	6.4	9.4	11.5	15.0	16.6
Malignant Tumors	39	32.8	7.0	14.8	14.3	14.8	16.3

† ALBUMIN

TABLE - IV

HAEMOGRAM NORMAL DOGS

Sl. No.	Sex	Age	Breed	Hb. G%	RBC. m/cmm	PCV. %	ESR. mm/1hr.	WBC. per cmm	N %	L %	M %	B %	E %
1.	F	Adult	N.D	11.5	5.96	33	1	9,700	67	31	1	-	1
2.	M	"	"	12.0	6.03	37	2	9,900	69	29	1	-	1
3.	M	"	"	13.5	6.23	39	1	9,600	68	27	2	-	3
4.	M	"	"	12.5	6.05	38	2	10,650	64	28	2	-	6
5.	F	"	"	12.0	5.86	35	3	11,800	71	29	-	-	-
6.	M	"	"	10.5	5.14	32	1	10,200	68	31	-	-	1
7.	F	"	"	11.0	5.58	34	1	9,800	62	36	2	-	-
8.	F	"	"	9.5	5.24	30	1	10,200	64	33	1	-	2
9.	M	"	"	12.0	6.21	35	1	11,900	67	30	1	-	2
10.	M	"	"	11.5	5.92	34	2	9,600	65	32	1	-	2
11.	F	"	"	11.5	5.2	30	1	9,800	60	37	2	-	1
12.	M	"	"	12.5	5.1	34	1	10,200	62	36	-	-	2
		MINIMUM		9.5	5.1	30	1	9,600	60	27	1	-	1
		MAXIMUM		13.5	6.23	39	3	11,900	71	37	2	-	6
		MEAN		11.75	5.71	34.25	1.42	9,446	66	32	1	-	2

F: FEMALE M: MALE N.D: NONDESCRIPT

TABLE V

HAEMOGRAM ASCITES DOGS

Sl. No.	SACOP No.	Sex	Age Y.- M.	Breed	Hb. G%	RBC. m/cmm	PCV. %	ESR. mm/1hr.	WBC/cmm	N %	L %	M %	B %	E %
1.	5294	F	1 - 0	N.D.	7.5	4.45	22	1	17,800	40	29	5	-	26
2.	6262	F	8 - 6	N.D.	10.0	5.27	32	12	15,050	60	28	8	-	4
3.	6410	M	1 - 0	N.D.	7.0	4.01	22	Less than one	14,300	78	8	4	-	10
4.	7066	F	3 - 0	Alsatian	11.0	6.04	32	1	16,550	77	16	2	-	5
5.	10902	M	2 - 6	Dachshund	4.5	2.16	15	5	18,400	71	28	-	-	1
6.	11085	F	2 - 0	N.D.	6.0	3.11	19	10	13,450	60	24	5	-	11
7.	11437	F	3 - 0	Alsatian	13.0	6.62	38	10	9,000	78	15	1	-	6
8.	11786	F	8 - 0	Alsatian	8.0	4.26	25	8	10,500	75	32	-	-	3
9.	12307	M	5 - 0	Alsatian	11.5	3.98	33	3	14,200	71	27	-	-	2
10.	12581	M	6 - 0	Dachshund	8.5	3.7	26	3	14,400	69	30	-	-	1
11.	12805	M	0 -10	N.D.	11.0	5.78	33	18	20,300	71	29	-	-	-
12.	13478	M	1 - 6	N.D.	10.5	4.04	22	2	12,600	79	30	-	-	1
				MINIMUM	4.5	2.16	15	1	9,000	40	8	1	-	1
				MAXIMUM	13.0	6.62	38	18	20,300	79	30	8	-	26
				MEAN	9.04	4.45	27	6	14,713	69	25	2	-	6

F: FEMALE M: MALE N.D.: NONDESCRIPT

TABLE - VI

PROTEINOGRAM NORMAL DOGS

Serial Number	Sex	Age	Breed	Albumin %	GLOBULINS %		
					Alpha	Beta	Gamma
1.	F	Adult	N.D.	46.19	10.68	22.44	20.69
2.	M	"	"	47.50	11.00	22.74	19.26
3.	M	"	"	45.49	12.86	22.13	19.52
4.	M	"	"	44.52	12.68	23.12	19.68
5.	F	"	"	43.62	11.17	24.47	20.74
6.	M	"	"	41.96	13.02	23.17	21.85
7.	F	"	"	48.96	10.82	22.16	18.06
8.	F	"	"	43.95	14.28	17.03	24.74
9.	M	"	"	38.92	12.02	21.09	27.97
10.	M	"	"	35.29	8.92	25.01	30.78
11.	F	"	"	38.23	11.16	23.52	27.09
12.	M	"	"	42.01	9.98	21.89	26.12
			MINIMUM	35.29	8.92	17.03	18.06
			MAXIMUM	48.96	14.28	25.01	30.78
			MEAN	43.05	11.54	22.39	23.55

F: FEMALE M: MALE N.D: NONDESCRIPT

TABLE VII

PROTEINOGRAM ASCITES DOGS

Sl. No.	SACOP No.	Albumin %	GLOBULINS %				Result
			Alpha 1 2	Total ALPHA	Beta	Gamma	
1.	5294	4.59	-	6.41	25.69	63.31	Died
2.	6262	22.77	-	9.75	32.14	35.34	Died
3.	6410	33.49	-	13.11	35.08	18.32	Discontinued
4.	7066	11.03	-	3.45	36.45	49.07	-do-
5.	10902	4.17	-	-	16.68	79.15	-do-
6.	11085	23.62	8.25+17.0	25.25	26.01	25.12	-do-
7.	11437	17.33	-	4.0	34.06	44.01	Died
8.	11786	28.64	7.28+ 9.89	17.17	21.82	32.37	Died
9.	12307	24.29	-	9.61	30.56	35.54	Discontinued
10.	12581	12.63	-	1.46	34.61	51.30	Died*
11.	12805	29.51	9.83+ 3.93	13.76	26.22	30.51	Discontinued
12.	13478	19.09	15.31+11.71	27.02	21.01	32.88	-do-
	MINIMUM	4.17	NIL	NIL	16.68	18.32	
	MAXIMUM	33.49		25.25	36.45	79.15	
	MEAN	19.26		10.92	28.41	41.41	

* Post-mortem could not be done

TABLE - VIII

PROTEINOGRAM ASCITES DOGS STUDY DURING THE COURSE OF THE DISEASE

Sl. No.	SACOP NO.	Date of Serum Collection	Albumin %	GLOBULINS %			
				Alpha 1 + 2	TOTAL ALPHA	Beta	Gamma
1.	11085	30-9-1966	23.62	8.25 + 17.0	25.25	26.01	25.12
		3-10-1966	7.06	- -	12.94	37.64	42.36
ii.	11786	21-10-1966	28.64	7.28 + 9.89	17.17	21.82	32.37
		18-11-1966	12.38	- -	14.28	30.47	42.87

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