ANALYSIS OF NORMAL DOG SERUM BY IMMUNOELECTROPHORESIS

Shin OKOSHI, Isamu TOMODA and Susumu MAKIMURA

*Department of Veterinary Internal Medicine and Parasitology,
Faculty of Agriculture, University of Tokyo, Tokyo*

(Received for Publication May 23, 1967)

Following the introduction of the moving boundary apparatus of TISELIUS in 1937, most numerous studies have been undertaken to gain insight into alterations of serum proteins in various diseases^{7,21}). The authors have also determined physiological and pathological changes of serum proteins in animals in order to obtain basic data on the clinical application of paper electrophoresis³⁰⁻³⁷). In the previous papers, it was observed that most of the diseases produced abnormalities in the serum protein patterns, which were nonspecific, and that the successful application of paper electrophoresis as an aid to the diagnosis of diseases was still premature. However, the data obtained from these studies suggested that the changes in serum proteins might be characteristic of the host's reaction to infection or injury. It is reasonable to presume that the serum protein patterns do contribute to a more complete knowledge of the injuriousness of the disease, the immunological manifestation, and others.

On the other hand, the immunoelectrophoretic method enables differentiation of serum proteins in combination with agar-diffusion and electrophoresis. Over 20 different components have been demonstrated in the serum of the normal human being and animal by this method¹¹). Immunoelectrophoresis of sera from healthy individuals has provided additional information on the number of serum proteins, the characteristic and distribution of antibodies, and the nature of immunoglobulins. Moreover, it has also some advantages in the studies of pathological sera and enables to expand the knowledge of qualitative and quantitative changes in blood proteins, paraproteinemia and dysproteinemia.

In the authors' laboratory, attempts have been made to apply zone electrophoretic methods and immunoelectrophoretic techniques to small animal practice. To identify the pathological serum components it was necessary to determine the patterns produced by normal dog and cat sera. There are many reports on the analysis of dog and cat serum proteins under normal conditions by the boundary and the zone electrophoretic methods. Most of them, however, are on limited studies and, to the authors' knowledge, no current immunoelectrophoretic studies on dog and cat sera are available. On the other hand, it has been known that the serum proteins are affected by age and that the newborn has maternal antibodies in those animals, but no studies have been made on the transmission of the γ -globulins from mother to young. In the present investigation, the normal dog serum proteins were analyzed by immunoelectrophoresis, and the transfer of the globulins from mother to neonatal puppy was also studied.

MATERIALS AND METHODS

Healthy adult and young dogs, over two months old, harboring no eggs of any intestinal parasites in the feces nor microfilariae in the circulating blood, were used as materials.

Jap. J. vet. Sci., **29**, 233 ~ 244 (1967)

In addition, newborn puppies at birth and puppies one to sixty days of age were used in the present study. All of them were born from healthy bitches that had manifested no clinical signs of any acute or chronic disease during pregnancy, gestation, and lactation. They showed steady and satisfactory growth. Most of the newborn and suckling puppies were found prenatally infected with roundworms, *Toxocara canis*, but all of them were used as materials, except those involved in heavy infection.

Blood samples were collected usually from the cephalic or the recurrent tarsal vein in the adult and young dogs, from the jugular vein in the puppies one to two months old, and by cardiac puncture from the newborn and suckling ones not more than 3 weeks of age. They were taken after starvation for about 12 hours, except in the newborn and suckling subjects.

Normal human blood was collected from a healthy individual.

The blood samples were allowed to stand and clot at room temperature for about 30 minutes and centrifuged at 3,000 rpm for 30 minutes. The resultant sera were stored at -20°C when not in use. Cellulose acetate, agar gel, and paper electrophoresis were performed on all serum samples either immediately after separation from the clot or within 7 days after freezing. The sera used for immunoelectrophoresis had not been frozen. Serum was discarded with hemolysis was present.

Antiserum against dog whole serum was prepared in rabbits by two inoculation series in the following manner. The initial series consisted of four subcutaneous inoculations of 2 ml of sample given over a period of 30 days. That is, a total of 4 ml of normal dog serum with an equal amount of Freund's complete adjuvant (Iatron Laboratories, Tokyo) was administered. The second series was composed of four subcutaneous inoculations which began 28 days after the last inoculation of the initial series. Eight milliliters of normal dog serum was given over a period of 4 weeks. The rabbits were bled on the 10th and 15th day after the final inoculation. All sera were preserved in Merthiolate (1:10,000) and stored at -20°C .

Rabbit antiserum against human whole serum was purchased from a commercial source (Behringwerke, AG., Marburg-Lahn, Germany).

Immunoelectrophoresis was performed by a modification of the method proposed by GRABAR and WILLIAMS¹⁰⁾, using a Fuji agar-electrophoretic apparatus (Fuji-Riken Co., Tokyo). Barbitol-sodium hydrochloric acid buffer was used at a pH of 8.6. The ionic strength of the buffer was 0.1 in both agar barriers and the electrode compartment of the cell, and 0.04 in agar on the plate. Plates, microscopic slides, were used with approximately 2.5 ml each of 1% Difco noble agar mounted on them as supporting medium. A total of 5 slides was placed in direct contact with the agar gel barriers. The slides were processed in the cell at room temperature by applying a current of 15 milliamperes per slide and cooling with an overlay of petroleum ether. The slides were removed from the cell after electrophoresis. Then antiserum troughs were made by removing cut agar strips with a pen point. The antiserum was added to the troughs, and the slides were placed in a moist chamber at room temperature for 24 to 72 hours to allow to precipitin arcs develop. The slides were read against a black background with reflected light.

To identify the precipitin arcs, the slides were stained as follows. At first, the slides were allowed to stand in saline solution for 5 to 7 days so that excess proteins might be removed from the gel. Then they were covered with a single sheet of moist filter paper so that no air might be trapped between the paper and the agar. They were dried overnight at 37°C , and then stained with Amidoschwartz 10 B. In addition to the routine staining with this dye, some of the slides were stained with Sudan black B for lipoproteins and with Schiff's reagent for glycoproteins. To demonstrate oxidase and peroxidase activity, paraphenylenediamine and o-dianisidine were used, respectively.

Simple agar gel electrophoresis¹⁹⁾ was carried out on slides in the same manner as immunoelectrophoresis, except that an antiserum trough had not been cut. After the electrophoresis, the slides were immediately immersed in methanol-acetic acid (9:1) solution for 5 minutes to fix the proteins, and then in warm methanol (40°C) for 20 minutes to remove the barbital contained in the agar gel. The subsequent procedure was carried out mostly in such manner as described in the method of immunoelectrophoresis.

Cellulose acetate electrophoresis²³⁾ was performed on 1 by 5 cm strips of OXOID (Oxo Ltd., London) using barbital buffer, pH 8.6, in a microelectrophoretic apparatus manufactured by the Johko-Sangyo Co. In this apparatus, as many as 28 samples of such strips could be handled at the same time. After moist strips were set on the bridge, samples (0.0008 to 0.001 ml) were placed carefully on the strips. Then a constant current with an intensity of 5 milliamperes per cm of the strip was applied at room temperature for about 45 to 60 minutes. After electrophoresis, the strips were stained immediately with Ponceau 3 R, rinsed through several (usually 5) changes of 3% acetic acid solution, and dried at room temperature or 37°C. The method of paper electrophoresis used was the same as mentioned previously by the authors³⁰⁾. The stained strips of paper and OXOID were scanned with the recording densitometer, Densicord (Photovolt Ltd., U.S.A.). The percentage of individual fractions were calculated by counting the number of teeth on the integrating lines of the Densicord patterns.

Protein concentrations were determined by using a commercial refractometer (Hitachi hand protein refractometer, model PRP, Hitachi Ltd.).

RESULTS

Immunoelectrophoresis of normal adult dog serum developed by anti-normal dog whole serum usually reveals at least 20 precipitin arcs. Chart 1 is a diagrammatical representation of all the precipitin lines observed in immunoelectrophoresis and the nomenclature proposed by CHORDI and KAGAN⁵⁾. The mobility of the fractions was calculated by plotting the centers of the precipitin arcs. Six groups, consisting of albumin and α_1 -, α_2 -, β_1 -, β_2 -, and γ -globulin, were differentiated by the mobility. In the α and β regions, however, determination of the exact position of each precipitin line was difficult on account of the large number of arcs. To clarify the true number of lines, each of the globulin fractions, which had been separated from one another by paper or cellulose-acetate electrophoresis in advance, was subjected to immunoelectrophoresis, which was developed by anti-normal dog serum. In consequence, the position and the number of precipitin lines were recognized more easily in each of the groups. By using these conventional methods, one line in the albumin fraction, 6 in the α_1 -globulin fraction, 6 in the α_2 -globulin fraction, 3 (occasionally 4) in the β_1 -globulin fraction, 3 (occasionally 4) in the β_2 -globulin fraction, and one in the γ -globulin fraction were observed in the normal adult dog sera.

On the other hand, 14 arcs of glycoprotein were exhibited by staining with Schiff's reagent (Chart 2, D) and 2 lipoprotein lines, α_1 -II and α_2 -IV, by staining with Sudan black B (Chart 2, F). By the benzidine test of normal dog serum mixed with hemoglobin, weak peroxidase activity was observed in three lines of the albumin, α_2 -I, and β_1 -II. Simple agar-gel electrophoresis of the same serum showed strong peroxidase activity in the region of β_1 -globulin fractions and weak peroxidase activity in the α_2 -globulin fraction (Chart 2, G and H). However, staining of normal dog serum with paraphenylenediamine did not show any definite precipitin line. Besides, the supernatants resulting from precipitation of sera with 4 volumes of 0.4% rivanol were also analyzed by electrophoresis and immunoelectrophoresis. It contained the bulk of the γ -globulins and of the transferrin. The zone-electrophoresis of the supernatant showed two fractions of β_2 - and γ -globulin, and

Table 1. Precipitin Lines in the Immunoelectrophoretic Pattern of Normal Adult Dog Serum, Staining Characteristics, and Comparison with Lines in That of Human Serum

Precipitin line in dog serum	Staining Characteristic	Corresponding line in human serum
Albumin	Glycoprotein Peroxidase activity	Albumin
α_1 -I	Glycoprotein	α_1 -glycoprotein
α_1 -II	Lipoprotein	α_1 -lipoprotein
α_1 -III	Glycoprotein	
α_1 -IV	Glycoprotein	
α_1 -V		
α_1 -VI		
α_2 -I	Glycoprotein Peroxidase activity	Haptoglobin
α_2 -II	Glycoprotein	
α_2 -III	Glycoprotein	
α_2 -IV	Lipoprotein	α_2 -lipoprotein
α_2 -V		
α_2 -VI		
β_1 -I	Glycoprotein	Transferrin
β_1 -II	Glycoprotein Peroxidase activity	Hemopexin
β_1 -III		
β_1 -IV		
β_2 -I	Glycoprotein	γ A
β_2 -II	Glycoprotein	
β_2 -III	Glycoprotein	γ M
β_2 -IV	Glycoprotein	
γ	Glycoprotein	γ G

Chart 1. Diagrammatic Representation of Immunoelectrophoretic Pattern of Normal Adult Dog Serum Developed against Rabbit Antiserum to Dog Whole Serum

The figure in parentheses gives the number of precipitin lines found in each mobility range.

distinct two precipitin arcs (β_2 -I and γ) and weak line (β_1 -I) were usually observed in the immunoelectrophoresis (Chart 2, I and J). The number of precipitin lines found in normal adult dog serum, their special staining characteristic, and their corresponding bands in normal human serum are listed in Table 1.

Changes in the serum proteins were scrutinized from the newborn to the adult. The most striking alteration with age was found in the γ -globulins. In precolostral newborn puppy sera, the total proteins, including albumin, were of far lower value and the relative concentration of α -globulin was higher than in young and adult dog sera, and no γ -globulin fraction was observed by electrophoresis (Chart 3). Compared immunoelectrophoretically with puppy serum collected 24 hours after the first feeding with colostrum

Chart 2. Diagrammatic Representations of Agar Gel Electrophoresis and Immunelectrophoresis of Normal Adult Dog Serum

A and B: staining of proteins with Amidoschwarz 10 B.
C and D: staining of glycoprotein with Schiff's reagent.
E and F: staining of lipoprotein with Sudan black B.
G and H: staining of normal dog serum mixed with hemoglobin with α -dianisidine.
I and J: staining of the supernatant resulting from precipitation of serum with rivanol with Amidoschwarz 10 B.
A, C, E, G, and I: agar gel electrophoretic patterns.
B, D, F, H, and J: immunoelectrophoretic patterns.

and adult dog serum, precolostral puppy serum taken at birth showed a lack of γ -globulin corresponding to γ G-globulin. A typical pattern of newborn puppy serum developed with rabbit anti-normal adult dog serum is shown in Fig. 1 A. In all sera collected 24 hours after birth, the γ -globulin fraction appeared in the electrophoretic pattern, and the distinct precipitin line of γ -globulin in the immunoelectrophoretic pattern (Fig. 1 B). When dog colostrum was subjected to electrophoresis and then allowed to diffuse against anti-dog whole serum, a very strong line corresponding to γ -globulin in the serum was found (Fig. 1 C). Colostrum globulin was electrophoretically similar to the γ -globulin component of serum. It was agreed that the γ -globulin of colostrum was antigenically identical with the γ -globulin of serum and that the appearance of γ -globulin observed in the serum of 24-hour-old suckling puppies was due to the absorption of γ -globulin by these puppies through the colostrum after birth. On the other hand, the significant amounts of the α -globulins, particularly the fraction corresponding to α_1 -glycoprotein, were demonstrated in precolostral newborn puppy sera, but those globulin fractions decreased rapidly after birth. In the

Chart 3. Examples of Cellulose Acetate Electrophoretic Patterns of Serum Proteins in Various Age Groups of Normal Dogs

Newborn: precolostral puppy. Adult: dog 3 years old. TP: Total protein value.

The arrow indicates the point of application of the sample.

of age, this line was observed when conditions were suitable, although it was very faint. In sera from young and adult dogs, this fraction was distinctly recognized.

newborn puppy sera, however, it may be seen that a number of α - and β -globulin fractions appear to present lower concentrations than arcs found in adult dog serum, and that several proteins seem to be entirely lacking.

The γ -globulin concentration did not change significantly in colostrum-fed newborn puppies up to 2 weeks of age, but exhibited a tendency to begin to decrease gradually about the 3rd week of age. In the sera of 1- to 2-month-old puppies, the γ -globulin fraction showed an excessive decrease. Reflecting the distinct decrease of γ -globulin found in the electrophoretic patterns, immunoelectrophoresis also revealed a short band of γ -globulin, as shown in Fig. 2. But γ -globulin began to rise in concentration at about 3 or 4 months of age and reached the adult level at about 6 to 8 months. In general, the γ -globulin component observed in adult dog serum by immunoelectrophoresis presented a strong and broad line starting with the γ -zone and running through the β_2 - and β_1 -fractions. In some of the adult dogs studied, however, a very long line running into the α -globulin area was occasionally found.

The β_2 -III line corresponding to γ M-globulin was not present either in newborn puppy serum.

In sera from some sucklings more than 2 weeks

DISCUSSION

The blood proteins of mammals can be divided into 5 to 7 components by moving-boundary electrophoresis^{7,8,20}. Many more components, however, can be delineated by electrophoresis and such immunoelectrophoretic techniques as designed to obtain higher resolution. Electrophoretic analysis of normal dog serum has been performed by numerous investigators and yielded the following results: Five to 9 components in moving boundary, up to 6 components in paper, 11 components in agar, and up to 16 components in starch gel electrophoresis⁸. In the previous paper³², one of the authors has also mentioned that paper electrophoresis using the conventional veronal buffer at pH 8.6 and 0.1 ionic strength divided the normal dog serum protein into 6 components, of which albumin was the fastest moving fraction and the other 5 components were arranged in the decreasing order of mobility as follows: α_1 -, α_2 -, β_1 -, β_2 -, and γ -globulins. The use of agar gel electrophoresis allowed the normal dog serum protein to be divided into 8 to 9 fractions including the same distinct 6 components as observed in paper electrophoresis and additional components separated in the α -globulin areas. Cellulose acetate electrophoresis could usually permit the dog serum protein to be separated into 7 fractions including an extra peak to appear between the albumin and α_1 -globulin fractions. On the other hand, the immunoelectrophoretic patterns developed with the authors' best antiserum to normal whole serum

indicated the presence of 22 antigenic constituents in normal adult dog serum.

Immunoelectrophoretic analysis has been conducted on the normal serum proteins in such animals as the cow^{12,16)}, horse¹³⁾, sheep^{5,29)}, pig³⁾, mouse^{6,39)}, rat^{2,15,22)}, mink^{27,28)}, and chick^{1,38)} in the recent years. In normal human serum, over 25 of the fractions precipitated by immunoelectrophoresis have been identified by various means. In animal sera, however, many of the precipitin lines observed have not been identified completely as yet. In general, a nomenclature chosen for identifying the precipitin lines is based on the electrophoretic mobility of the protein and the terminology employed in moving-boundary electrophoresis. Studying the analysis of normal sheep serum by immunoelectrophoresis, CHORDI and KAGAN⁵⁾ adopted a slight modification of the nomenclature described above, and identified the strongest and most reproducible lines and arcs that presented some stain character for identification in each electrophoretic region as arc number one in each zone. POTTER and DIXON²⁸⁾ also used the same nomenclature by CHORDI and KAGAN⁵⁾ to designate various fractions found in normal mink serum. On the other hand, SILVERSTEIN et al.²⁹⁾ who performed investigation limited to identification of the β_2 - and γ -areas in adult sheep serum, labelled the arcs as β_2A -, β_2M -, and γ -globulins in accordance with similar preparations from human serum. In the present paper, therefore, the nomenclature proposed by CHORDI and KAGAN⁵⁾ was adopted for identification of precipitin lines. The nomenclature, however, was a simple and direct method by which a component may readily be identified, but did not take precedence over specific naming procedures.

The staining of the precipitin lines of normal adult dog serum with Sudan black B demonstrated 2 lipoprotein fractions. The major part of the protein bound fats was a fast component corresponding to α_1 -lipoprotein which had migrated a little slower than albumin. Analysis by paper electrophoresis indicated that it belonged to α -lipoprotein. The minor component corresponding to α_2 -lipoprotein existed between the α - and β -globulin zones and appeared to be β -lipoprotein when analyzed by paper electrophoresis. The lipoprotein stain of the minor component, α_2 -IV, was weak in comparison to the α_2 -lipoprotein of the human serum. The lipoproteins of human serum seemed to be quantitatively different from those of dog serum. In human serum, the major part was β -lipoprotein and the minor part α -lipoprotein in the agar gel or paper-electrophoresis. In dog serum, however, the major lipoprotein was α -lipoprotein. One of the authors³⁴⁾ reported previously that the mean percent values of the dog serum lipoproteins determined by paper electrophoresis were 72.0 and 28.0 for α - and β -lipoprotein, respectively. In general, species variations exist not only in the type of lipoproteins but also in the absolute amount. LEWIS et al.¹⁷⁾ reported that the lipoproteins in the S_f 1-15 class (α -lipoproteins) were over 200 mg/dl in dogs and about 150 mg/dl in human serum, and that the lipoproteins in the S_f 15-40 class (β -lipoproteins) were 50 mg/dl in dog and over 200 mg/dl in human serum.

Hemoglobin-binding proteins or haptoglobins have been found in the plasma of human and many animals. LIANG¹⁸⁾ reported that free hemoglobin appeared as a single spot with almost the same mobility as β -globulin or fibrinogen, but when it was mixed with plasma from the same species the number of benzidine staining spots and their mobility were characteristic of the species. In dogs, he mentioned that free hemoglobin formed completely with α_2 -globulin, but when hemoglobin was mixed with the plasma the electrophoretic pattern showed 2 staining spots in the α_2 - and β_1 -globulin regions by benzidine test. In the present study, simple agar gel electrophoresis of dog serum with hemoglobin added showed peroxidase activity in 2 fractions of α_2 - and β_1 -globulin, but the immunoelectrophoresis showed peroxidase activity by 3 arcs of albumin, α_2 -I, and β_1 -II.

Studies were made electrophoretically on the relationship between the serum proteins and the age groups. No γ -globulin was contained in precolostral newborn puppy serum,

whereas a considerably large amount of γ -globulin appeared in suckling puppy serum. Some papers were published on the detection and description of changes in the serum proteins during embryonic and postnatal development in human and animals^{7,8}. The transfer of immunoglobulins from mother to young occurs by different mechanisms according to species, and can be roughly correlated with the type of placentation. In the rabbit, guinea pig, monkey, and human, all newborn passive immunity is obtained by the transplacental route. In the newborn of these species, the serum contains a normal amount of γ -globulin when analyzed electrophoretically. On the other hand, it is generally agreed that in calves, foals, lambs, kinds, and piglets, the serum contains little or no γ -globulin at the newborn stage, but a high concentration of γ -globulin within a few hours after colostrum is fed. In rats, mice, cats, and dogs, however, transmission of antibody from mother to young occurs both before and after birth. Accordingly, the newborn young of these species have a small but significant amount of maternal antibody before they begin to be suckled, but most of their passive immunity is derived from the colostrum and milk after birth.

ASOFSKY et al.¹³ and TUREEN et al.³⁸ studied the development of serum proteins in chick embryos and in chickens immunoelectrophoretically, and observed that the γ -globulins found in embryonic and neonatal chicken sera consisted of 3 fractions corresponding to γ A-, γ M-, and 7S γ -globulin, respectively. Umayahara and Migita³⁹ reported that fetal mouse serum contained a small amount of 7S γ -globulin, but neither γ A- and γ M-globulin. However, Porter²⁷ mentioned that the mink, which has essentially the same hemochorial type of placenta as the dog, was born without a detectable amount of γ -globulin in the serum, and that it acquired this globulin subsequently from the mother via the colostrum and milk. In general, the transmission of antibody from mother to young occurs before birth in dogs. It is observed, however, that the antibody transmitted through maternal serum is very low in titer, and that only 3 to 5% in titer of the maternal antibody against canine distemper⁹ or infectious hepatitis⁴⁰ virus is present in puppy serum at the time of birth. Carmichael et al.⁴¹ showed that the newborn puppy could reach the same level of antibody titer as the dam within 72 hours after birth. Harding et al.¹⁴ reported that the passive antibody transfer in the cat was similar to that in the dog, but that a much higher antibody concentration crossed the placental barrier in the cat than in the dog. However, lack of γ -globulin in newborn puppy serum was demonstrated by various electrophoretic analyses, as mentioned above. Moreover, even in newborn kitten serum, γ -globulin was absent²⁴. Although antibodies are usually associated with the γ -globulins, all the γ -globulins are not antibodies. All antibodies are not found in the γ -globulins. Certain antibodies are detected from the other electrophoretic fractions, even from the albumin. Therefore, it is reasonable to presume that the lack of γ -globulin observed in fetal and newborn puppy sera was not inconsistent with the literature which contains several references to the placental transmission of antibody.

The precipitin arc of the β_2 -III fraction which probably corresponds to γ M-globulin (19S γ -globulin) was absent in newborn and suckling puppies up to 14 days of age, but was present in adult dogs. Generally, this fraction does not migrate freely by immunoelectrophoresis. Considerable material remains around the site of its origin. It can be demonstrated in normal serum when conditions are suitable. Certainly, it is evident that the immunoelectrophoretic preparation needs optimal conditions for the precipitation of electrophoretically separated protein fractions, and that separation of the fractions depends on the medium used, the pH and ionicity of the buffer, the voltage and amperage used for electrophoresis. Accordingly, the absence of an arc in the immunoelectrophoretic traces does not necessarily represent the absence of the fraction. It is observed, however, that 19S antibodies, at least those in man, do not cross the placenta to any great extent.

UMAYAHARA and MIGITA³⁹⁾ also reported the absence of γ M-globulin in fetal and newborn mouse sera. From these results, it seemed evident that no neonatal puppy could synthesize 19S γ - or 7S γ -globulin and that none of these proteins were transmitted from to fetus in any appreciable amount.

The precipitin arcs of the 7S γ_1 - and 7S γ_2 -globulin fractions which appear about 4 days after birth are very distinct in the mouse³⁹⁾. PORTER²⁷⁾ stated that the serum of the mink kit contained 24 to 30% of the maternal serum concentration of γ -globulin at 2 days of age, and 60 to 80% at 8 days. In the present study on the development of the serum proteins in dogs from the newborn to the adult stages, a somewhat similar change was also observed in the γ -globulin fractions. When the colostrum was analyzed immunoelectrophoretically, it was evident that the appearance of γ -globulins in suckling puppy serum was due to the absorption of the γ -globulins by the puppy from the colostrum after birth. In general, the absorption of the γ -globulins through the gut is continued for 10 days in the dog and mink and 16 days in the mouse⁸⁾. In young puppies, the γ -globulin fractions decreased subsequently at about 2 weeks of age and reached very low levels at one to two months. Such physiological decrease, therefore, seemed reasonable to be explained as follows: The γ -globulins transmitted from the mother by transplacental transfer were absent or very little, but a significant amount of the γ -globulins occurred within a few hours after birth as the result of absorption of γ -globulins contained in the colostrum. On the other hand, the capacity of producing γ -globulins and antibodies did not develop for some time after birth. The γ -globulins and antibodies contained in the puppy serum were continually reduced by metabolic decay and diluted, to some extent, due to growth of the puppy. In consequence, the γ -globulins began to decrease gradually at 10 days of age, when the absorption of the γ -globulins from the gut became impossible.

Infection with such helminthes as *Toxocara canis* and *Ancylostoma caninum* is very common in dogs. Under natural conditions, therefore, it may fairly be said that infection with some parasites is probably unavoidable during the fetal life and that it is impossible for puppies to grow into adults without being infected with various parasites. No remarkable changes were noticed electrophoretically in the serum proteins in the case of slight infection. In view of these results, newborn and suckling puppies infected prenatally with *Toxocara canis* were used as materials, except puppies with heavy infection. It is impossible, however, to discuss the helminthic infection without taking immunity into consideration. In general, the immunity that develops against parasitic helminths is similar to that which develops against bacterial and viral organisms. Accordingly, it is felt undoubtedly that the serum protein patterns, especially the immunoglobulin patterns, observed in puppies may be affected to some extent by the helminthic infection. It was believed, however, that the changes of serum protein observed in the present study were not grossly in error, but that they showed the rough tendency of the γ -globulin fractions to develop from the newborn to the adult stage in dogs, even if they were not absolute changes.

The glycoproteins of serum are generally affected by age. It is agreed that α_2 -glycoprotein and hexosamine are very low in concentration in the newborn human infant, and that the capacity of producing α_2 -serum protein, probably haptoglobin, does not develop for some time after birth. On the other hand, a significant amount of α_1 -glycoprotein corresponding to fetuin^{25,26)} is contained in the serum of the newborn calf, foal, lamb, and kid. In the results of the previous and the present studies, it was apparent that fetal and newborn puppy sera contained significant values of relative concentrations of α - and β -globulin fractions. The immunoelectrophoretic patterns also showed distinct lines of some glycoprotein fractions, probably α_1 -glycoprotein, transferrin, hemopexin, and the like. On the contrary, a number of α - and β -globulin fractions appeared to be present in low concentration and several proteins seemed to be entirely lacking. Since successful identifica-

tion of the arcs in these regions has not been achieved consistently, it would be necessary to investigate further the age variation in the serum glycoproteins.

SUMMARY

Normal dog serum was analyzed by electrophoretic and immunoelectrophoretic methods. Electrophoretic analysis of normal adult dog serum yielded 6 to 7 distinct components. In normal adult dog serum, at least 20 antigenic constituents were demonstrated by immunoelectrophoresis. The nomenclature proposed by CHORDI and KAGAN⁵⁾ was adopted for identification of precipitin lines. Some special staining characteristics of the precipitin lines were investigated.

In addition, the sera of dogs from the newborn to the adult stage were analyzed. The most striking alteration with age was found in the γ -globulins. It was observed that the serum of the precolostral newborn puppy was characterized by the complete absence of γ -globulin pattern and arc. In the colostrum-fed puppy, a distinct pattern and arc of γ -globulin appeared within 24 hours after birth. In dog colostrum, a very strong line corresponding to γ -globulin in the serum was found. It was agreed that the appearance of γ -globulin in suckling puppy serum was due to the absorption of γ -globulin from the colostrum after birth. Thereafter, the γ -globulin fraction began to decrease gradually at about 2 to 3 weeks of age and reached very low levels at one to 2 months of age. On the basis of these results, the transmission mechanism of γ -globulins from mother to young was discussed.

REFERENCES

- 1) ASOFSKY, A., TRANKA, Z. and TJORBECKE, G. J. (1962): *Proc. Soc. exp. Biol. (N.Y.)*, **111**, 497~499.
- 2) BENJAMIN, D. C. and WEIMER, H. E. (1963): *J. Immunol.*, **91**, 331~338.
- 3) BRUMMESTEDT-HANSEN, E. and HIRSCHFELD, J. (1961): *Acta vet. scand.*, **2**, 317~326.
- 4) CARMICHAEL, L. E., ROBSON, D. S. and BARNES, E. D. (1962): *Proc. Soc. exp. Biol. (N.Y.)*, **109**, 577~581.
- 5) CHORDI, A. and KAGAN, I. G. (1964): *J. Immunol.*, **93**, 439~445.
- 6) CHORDI, A., WALLS, K. W. and KAGAN, I. G. (1964): *J. Immunol.*, **93**, 1034~1044.
- 7) DIMOPOULLOS, G. T. (1963): In: *Clinical biochemistry of domestic animals*, edited by CORNELIUS, C. E. and KANEKO, J. J., pp. 109~201, New York and London, Academic Press.
- 8) ENGLE, R. L. and WOODS, K. R. (1960): In: *The plasma proteins*, Vol. II, edited by PUTNAM, F. W., pp. 183~265, New York and London, Academic Press.
- 9) GILLESPIE, J. H., BAKER, J. A., BURGHER, J., ROBSON, D. and GILMAN, B. (1957): *Cornell Vet.*, **48**, 103~126.
- 10) GRABAR, P. and WILLIAMS, C. A. (1955): *Biochim. biophys. Acta (Amst.)*, **17**, 67~74.
- 11) GRABAR, P. and BURTIN, P. (1964): *Immunoelectrophoretic analysis, applications to human biological fluids*, Amsterdam, Elsevier Pub. Co.
- 12) GRAVES, J. H. (1963): *J. Immunol.*, **91**, 251~256.
- 13) HANSEN, J. B. (1965): *Amer. J. vet. Res.*, **25**, 1706~1771.
- 14) HARDING, S. K., BRUNER, D. W. and BRYANT, I. W. (1961): *Cornell Vet.*, **51**, 535~539.
- 15) HASE, T. and MAHIN, D. T. (1965): *J. Immunol.*, **94**, 191~196.
- 16) JANSEN, K. (1963): *Acta vet. scand.*, **4**, 64~84.
- 17) LEWIS, L. A., GREEN, A. A. and PAGE, I. H. (1952): *Amer. J. Physiol.*, **171**, 391~400.
- 18) LIANG, C. C. (1957): *Biochem. J.*, **66**, 552~558.
- 19) MIGITA, S. (1966): *Jap. J. clin. Path.*, Suppl. 11, 19~32 (in Japanese).
- 20) MOORE, H. D. (1945): *J. biol. Chem.*, **161**, 21~32.
- 21) MOORE, H. D. (1959): In: *Electrophoresis, Theory, methods, and applications*, edited by BIER, M., pp. 369~425, New York and London, Academic Press.

- 22) NEUHAUS, O. W., HAUEZ, R. and BISERTE, G. (1964): *Arch. Biochem.*, **106**, 159~166.
- 23) OGAWA, Y. (1966): *Jap. J. clin. Path.*, Suppl., **11**, 46~63 (in Japanese).
- 24) OKOSHI, S., TOMODA, I. and MAKIMURA, S. (1967): *Jap. J. vet. Sci.*, **29**, (in press).
- 25) PEDERSEN, K. O. (1944): *Nature (Lond.)*, **154**, 575.
- 26) POLSON, A. (1943): *Nature (Lond.)*, **152**, 413~414.
- 27) PORTER, D. D. (1964): *Proc. Soc. exp. Biol. (N.Y.)*, **119**, 131~133.
- 28) PORTER, D. D. and DIXON, F. J. (1966): *Amer. J. vet. Res.*, **27**, 335~338.
- 29) SILVERSTEIN, A. M., THORBECKE, G. J., KRANER, K. L. and LUKES, R. J. (1963): *J. Immunol.*, **91**, 384~395.
- 30) TOMODA, I. (1962): *Jap. J. vet. Sci.*, **21**, 337~348 (in Japanese).
- 31) TOMODA, I. (1962): *J. vet. Med. (Japan)*, No. 334, 967~973 (in Japanese).
- 32) TOMODA, I. (1962): *J. Japan vet. med. Ass.*, **15**, 404~410 (in Japanese).
- 33) TOMODA, I. (1962): *J. Japan vet. med. Ass.*, **15**, 481~485 (in Japanese).
- 34) TOMODA, I. (1963): *Jap. J. vet. Sci.*, **25**, 5~19 (in Japanese).
- 35) TOMODA, I. (1963): *Jap. J. vet. Sci.*, **25**, 67~91 (in Japanese).
- 36) TOMODA, I. (1963): *Jap. J. vet. Sci.*, **25**, 177~185 (in Japanese).
- 37) TOMODA, I. (1963): *J. vet. Med. (Japan)*, No. 357, 545~550 (in Japanese).
- 38) TUREEN, L. L., WARECKA, K. and YOLING, P. A. (1966): *Proc. Soc. exp. Biol. (N.Y.)*, **122**, 729~732.
- 39) Umayahara, H. and Migita, S. (1965): *Physico-chem. Biol. (Japan)*, **11**, 76~77 (in Japanese).

免疫電気泳動法による犬血清蛋白の分析

大越 伸・友田 勇・牧村 進

東京大学農学部家畜内科学及寄生虫病学教室

(昭和 42 年 5 月 23 日受付)

従来、著者らは、各種の疾病時における家畜血清蛋白の動態に関する臨床的意義を、病的状態や障害に対する生体反応反映の面に求めて、検討を加えてきた。最近、血清蛋白異常の意義をより明確にするための一助として、免疫電気泳動法によって、病的血清の動態を追究している。いうまでもなく、病的動態を明確にするためには、健康時の蛋白像を明らかにしておかねばならない。そこで、まず健康犬血清蛋白について、濾紙、セルローズアセート膜、寒天の各種電気泳動法、および免疫電気泳動法によって、若干の検討を行なった。

セルローズアセート電気泳動法による健康成犬の血清蛋白像としては、通常明瞭な 7 分画が観察され、特に α -globulin の分離が鮮明であった。寒天電気泳動法では、濾紙電気泳動法よりも分離能力がすぐれ、明瞭な 6 分画と若干の subfraction を含む 8~9 分画を認めることができた。一方、著者らの家兔抗犬全血清に対する健康成犬血清の免疫電気泳

動では少なくとも 20 本の沈降線を認めた。これらの沈降線の命名については、CORDI らの方法に準じて行なった。Schiff 試薬によって、最高 14 本の glycoprotein 沈降線を検出し、また、Sudan black B による lipoprotein 染色では、2 本の沈降線が確認された。さらに、hemoglobin を加えた血清に対する o-dianisidine 染色では、albumin を含めて 3 本の沈降線に peroxidase activity を認めた。Paraphenylenediamine による染色では、人血清は ceruloplasmin を検出できたが、犬血清は何ら反応を示さなかった。これら特異染色性や沈降線の位置などを基に人血清免疫電気泳動像と比較した。

哺乳前の新生児から成犬に至る犬血清蛋白の免疫電気泳動所見では、 γ -globulin の動態に最も著しい変動を認めた。哺乳前新生児血清では、 $\gamma(\gamma G)$ 沈降線は欠如している。しかし、24 時間目血清には、全例に明瞭な γG -globulin の出現がみられた。初乳血清の泳動所見から、この γ -globulin の出現は、初

乳から吸収されたものであることは明らかである。減少は次第に回復し、6~10 カ月令では、ほぼ成犬
2 週令以後、 γ -globulin は次第に減少して、1~2 カ月令では極端な減少を示した。3 カ月令以後、この
値に達した。

EXPLANATION OF PLATES

PLATE I

Fig. 1. Immunoelectrophoresis of normal dog sera and colostrum.

A, precolostral newborn puppy serum vs. anti-normal adult dog serum; B, colostrum-fed puppy (24 hours of age) serum vs. anti-normal adult dog serum; C, colostrum vs. anti-normal adult dog serum; and D, serum of dam vs. anti-normal adult dog serum.

PLATE II

Fig. 2. Immunoelectrophoresis of normal dog serum developed with rabbit antiserum to normal adult whole serum.

A, precolostral newborn puppy; B, colostrum-fed puppy 1 day old; C, puppy 7 days old; D, puppy 14 days old; E, puppy 21 days old; F, puppy 1 month old; G, young dog 3 months old; H, young dog 6 months old; I, young dog 10 months old; and J, adult dog 3 years old.