EVALUATION OF THE BROMSULFALEIN LIVER FUNCTION TEST IN THE RAT AND THE DOG

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E. JOHN LARSON

A THESIS

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(An Abstract)

The bromsulfalein (BSP) liver function test has had wide acceptance as a diagnostic aid in human medicine (Maclagan, 1956) and it has been applied to a limited extent in animals in both laboratory and field. In the studies here reported the results of the BSP test in the dog and in the rat have been correlated with various hepatopathies rather than with the results of other hepatic function tests as has often been done previously.

Hepler's (1952) procedure for determining serum concentration of dye was followed in the dog but in the rat the recommended volumes were halved. Sixty-five albino rats and eighty-five purebred and crossbred dogs were subjected to the evaluation. Dye retention was determined and gross pathologic examination made in normal rats and in those administered chloroform or pyrazolidin-3-one, 1-methyl-5-phenyl. Dye retentions were determined and gross and microscopic pathologic evaluations were made in: normal control dogs, dogs administered carbon tetrachloride, dogs receiving five other drugs, dogs given canine infectious hepatitis virus, dogs ill with canine distemper, and dogs with no other sign of disease than fever.

The BSP test was unreliable in the rat under the conditions of the experiment.

The normal limit of dye retention for the procedure was determined in the dog. Reciprocal agreement between retained BSP and cytological hepatic alteration in the dog was inconsistent in the lower range of

retention (below 5%). The two factors were generally but not uniformily correlative when the serum BSP was elevated. The BSP test was most correlative when the serum BSP was elevated. The BSP test was most correlative in acute hepatopathies. BSP test values from dogs used in drug toxicopathological investigations require cautious interpretation. The procedure appears to have questionable value in the differential diagnosis of canine infectious hepatitis and canine distemper complex because of the frequency of hepatic involvement in the latter disease. Lesions, other than hepatic, appeared inconsequential in relation to BSP retention. Fever, per se, in this study, had no apparent effect on the clearance of the dye from the blood stream.

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INTRODUCTION

Hepatic dysfunction in animals, as in man, arises from manifold causes, may or may not present a diagnostic picture, and can be significant or insignificant prognostically. It is of little moment, from the standpoint of the animal's welfare, whether the hepatopathy is primary or secondary; the principle consideration is the type and extent of the hepatic damage. This fact continues to present, as it has in the past, difficult diagnostic and prognostic problems to the clinician. The greatest obstacle to accurate prognostication lies in the phenomenal inherent functional reserve and regenerative powers of the liver. A severely damaged organ may reflect little clinically that indicates the seriousness of the condition and then, only too late, cause the development of indicative symptoms when the reserve powers are exhausted. Likewise, unsuspected hepatopathy, undetected because of the reserve, occurring with systemic or primarily non-hepatic diseases, may materially alter the effects of therapy and accuracy of prognosis. Conversely, the problem is further complicated by diseases whose signs mimic those of primary hepatopathy and require differentiation.

Inasmuch as these problems are not confined to veterinary medicine but are equally problematical in human medicine, an extensive battery of clinical laboratory procedures has been developed in the latter field to aid in differential diagnosis. Maclagan (1956) enumerates four factors determining the choice of tests in man: detection of liver damage in the absence of jaundice; differential diagnosis of jaundice; prognosis; and detection of excessive hemolysis. No single test has been developed to provide the requisite

information. The need has resulted in numerous laboratory manipulations on blood and urine for these purposes. The greatest concentration of tests centers on hepatic activities related to the following: biliary excretion, the metabolism of carbohydrates, proteins, amino acids and lipids, and detoxification.

The reasons for performing tests for hepatic dysfunction are as valid and as purposeful in lower animals as in man. Of the four factors requiring a differential test, from the practical standpoint, detection of liver damage in the absence of jaundice has received the most emphasis in veterinary practice. The majority of tests used in practice and in the laboratory are designed for this purpose. While large animals are subject to a great variety of hepatic disorders induced by viruses, bacteria, parasites, poisonous plants, and chemicals, and, would seemingly have a high requirement for tests to aid in differential diagnosis, in reality it is in cyniatrics that hepatic tests find their greatest utilization. Minimal restraint problems, economic feasibilities, and availability of laboratory facilities account for their frequent use in small animals.

The criteria for an ideal routine clinical laboratory procedure are dependability and simplicity. A dependable test requires that normal values lie in a reasonably narrow range and are consistently reproducible. Abnormal values should lie beyond the normal range with little or no overlapping and reflect the degree of malfunction. Simplicity, both in technique and apparatus, reduces the variables inherent in complicated procedures and tends toward closer reproducibility of results in different laboratories and among personnel. Such tests are rare. Many methods, however, approach the ideal and,

when used intelligently, are of value in estimating the over-all clinical picture. In this category are a number of methods for determining hepatic dysfunction including the bromsulfalein (BSP) liver function test. This determination measures the hepatic function of biliary excretion.

Since its development in 1925, the BSP function test has been modified by various investigators and has found wide acceptance in human medicine. It is considered the test of choice for demonstration of liver damage without jaundice and gives a higher percentage of positive results in cirrhosis than any other single test (Maclagan 1956). As a corollary, investigators applied the test to animals, in both laboratory and field. It has proven a valuable aid in experimental investigation and a body of literature has accumulated testifying to its usefulness. However, it has not found wide use in veterinary practice. Coffin (1953) probably states the concensus when he points out that the test is workable but possibly too exacting in its application for general use from an economic standpoint. It is equally true that it has not been applied extensively enough, especially in the dog, to fully elucidate its worth.

Perusal of the pertinent literature reveals that the preponderance of work has been concerned with correlation of individual tests rather than relationship of an individual test to manifold hepatopathies. A study to evaluate the BSP liver function test in rats and dogs with liver damage of multiple etiologies was deemed feasible. Further justification for this study, besides the practical application in the dog from the veterinary clinician's standpoint, arises from the utilization of large numbers of these animals in experiment-

al projects. In the rat, a widely used experimental animal, this information would allow following an induced disease or toxicological course without recourse to sacrifice. In the dog, it would aid in: a) screening dogs for test, b) following experimental alteration without the need for sacrifice, and c) confirming or refuting suspected hepatic malfunction in toxicopathology or other studies.

The objectives of these studies were:

a) To determine whether the method to be described was adaptable to the rat as a routine procedure and to evaluate its dependability in experimental hepatopathies.

b) To determine, in the dog, what correlation exists between the percentage of dye retention, and the type and extent of hepatic alteration.

Accomplishment of these purposes would require that the test be performed in intact rats and dogs to define the limits of normal dye retention, followed by estimation of retention in known or suspected hepatically altered animals. Correlation of retention and lesions would entail submission of the animals to a variety of known or suspected hepatotoxic agents, and the performance of the test in those with spontaneous diseases. In both instances, sacrifice of the subject, and gross and microscopic observations of the liver would be requisite.

As the work progressed, it was deemed necessary to broaden the scope of the studies to include the evaluation, in the dog, of the effect of fever and the role of pathologic alterations in other organs and tissues, especially the reticulo-endothelial system, on the retention of bromsulfalein.

REVIEW OF THE LITERATURE

The superiority of the bromsulfalein liver function test over other determinations for the early detection of hepatic dysfunction in animals has been demonstrated. Casal and Olitsky (1946), in an evaluation of bromsulfalein retention, blood bilirubin concentration, thymol turbidity, plasma coagulation time, and vaginal smear cytology in Swiss mice, found bromsulfalein retention the most dependable in indicating induced hepatic lesions. The fact that rats were unable to eliminate bromsulfalein normally even when thymol turbidity, cephalin flocculation, colloidal gold retention, serum albumin or globulin levels seldom showed change, was reported by Linder et al. (1953). When compared to serum phosphatase, prothrombin time and intravenous galactose tests, the BSP test was the most sensitive (Drill and Ivy, 1944) in dogs whose livers were damaged by carbon tetrachloride. Hoerlein and Green (1950) concluded that in suspected clinical cases of hepatosis in dogs, bromsulfalein retention was more sensitive in measuring dysfunction than methylene blue chloride, icterus index or the van den Bergh reaction. Comparing the bromsulfalein excretion test, serum alkaline phosphatase level and the serum protein faction levels, Gornall and Bardawell (1952) concluded that bromsulfalein retention probably reflected with fair accuracy the expected hepatotoxin-produced disturbance of function. When the results of the cephalin flocculation, thymol turbidity, van den Bergh and bromsulfalein retention tests were evaluated in dogs, Noyan (1949) found the latter the most sensitive in the detection of hepatic damage. In their work in dogs Svirbely et al. (1946)

demonstrated that of eleven different procedures the earliest indication of injury to the liver from xylidine was given by bromsulfalein retention.

The fate of the dye after injection has been investigated extensively. Mills and Dragstedt (1936) investigated the role of the reticulo-endothelial system in the clearance of bromsulfalein from the blood stream in dogs. In splenectomized dogs and in dogs in which the reticulo-endothelial system was blocked by India ink and saccharated iron oxide, they reported mild transient increase in retention of the dye from splenectomy and moderately elevated values following single and multiple injections of India ink and saccharated iron oxide. Continuous infusions of the two agents resulted in marked retention, especially with India ink. Their work confirmed the earlier observations of Klein and Levinson (1933). These latter investigators reported a slight but definite slowing of dye clearance from the blood in splenectomized dogs. India ink-injected dogs exhibited Kupffer cells laden with ink but the splenic endothelial cells were not so well blocked. Bromsulfalein retention thirty minutes after the ink injection resulted in a 70 to 100% retention at five minutes and 25 to 50% at the thirty minute sampling but normal values in three to four days after injection. Mills and Dragstedt (1938) also determined the rate of removal of the dye from the blood stream of normal dogs. Using a 2 mg./kg. dose, they found 85 to 90% was removed in five minutes and the remainder in thirty minutes. The same results were obtained with 5 mg./kg. Blockage of the reticulo-endothelial tissue resulted in 30 to 80% retention at five minutes and 10 to 50% at thirty minutes. In their experiments,

ether or ether and barbital anesthesia in twenty-nine dogs caused no increase in retention. Ligation of the bile ducts produced no appreciable reduction in dye clearance but administration of Decholin, a cholegogue, resulted in marked retention which was most notable in the first five minutes. Wirtz et al. (1951), working with cholecystectomized dogs which also had gastric and duodenal fistulae, had been treated with carbon tetrachloride, and had received 2 mg./kg. of bromsulfalein intravenously, hypothesized that impaired capacity of the liver to excrete the dye was first manifested by delay in transfer from the hepatic cells to the lumens of bile canaliculi. The removal from the blood was initially unaffected but with increasing functional damage the hepatic cells become increasingly saturated with dye which resulted in inability to remove the dye from the blood.

Cantarow and Wirtz (1941) reported 50 to 83% of bromsulfalein was excreted in the bile in the first hour and 67 to 100% within two hours in the dog and man. Cohn et al. (1947), employing constant intravenous infusion in dogs, found 31 to 65% of the dye was recovered in the bile. They calculated that in the absence of the liver, gastro intestinal tract and kidneys the peripheral tissue was capable of removing the dye 25 to 30% as rapidly as an intact animal. Using liver slices and perfused livers of rats, in the absence of bile formation, Bauer and Pessotti (1949a) suggested that bromsulfalein uptake in their experiments was independent of metabolic processes and that damage to the vascular bed in perfused livers resulted in reduction of uptake of the dye from above 90% to 10 to 40%. In the intact rat, they found carbon tetrachloride and India

ink readily reduced the removal of bromsulfalein from the blood stream; males cleared the dye more rapidly than females. Utilizing the continuous infusion technique in dogs, these investigators (1949b) determined that 77 to 89% of dye leaving the blood stream of normal dogs was extracted by the liver. Carbon tetrachloride-treated animals showed extremely low bromsulfalein concentration in the bile. In all cases, they demonstrated storage of the dye in the liver with the largest amount in the parenchymal cells. However, 11 to 23% was not accountable in the liver. Bauer et al. (1950) tagged bromsulfalein with S^{35} and made nearly quantitative recoveries of it from the liver, blood, and bile. S^{35} activity was recovered in bile dye from four fractions different from bromsulfalein, none of which was colorless. The intravenous infusion of bromsulfalein into dogs with portal and hepatic vein cannulae led Pratt et al. (1952) to conclude that loss of dye in urine, restoration of dye to the blood stream by intestinal absorption or lymphatic return was insignificant; the dye concentration of hepatic vein plasma sample was fairly representative of the concentration of total hepatic blood flow. Recovery of 30 to 50% of the dye in the urine was reported by Giges et al. (1952).

Linder et al.(1953) adapted the mouse test of Casals and Olitsky to the rat. The dye was administered intraperitoneally at the rate of 0.05 mg./gram of body weight and the blood sample collected in thirty minutes by severing a carotid artery. The determination was made using a Unicam spectrophotometer after treating the sera with 0.05 ml. of 10% HCl (blank) and 0.05 ml. of 10% NaOH, and the readings obtained at 556 mu. Using data from thirty-three normal

rats, they found that retention was unlikely to exceed 2.36 mg.%. In rats with necrosis of the liver produced by diet, they recorded retentions as high as 10.7 mg.%. With doses of 5 mg./100 grams of body weight, Giges et al. (1952) reported normal retentions of 1% or less in Sprague-Dawley rats using the method of Gaebler. By the same method of determination but injecting the bromsulfalein at the level of 5 mg./kg., they observed that retention of more than 5% was abnormal in dogs.

Drill and Ivy (1944) found normal retention betwee 2 and 12% in dogs when the dye was injected at 5 mg./kg. with a thirty minute serum sample, but using 2 mg./kg. standards. They regarded retentions over 15% as abnormal and recorded values as high as 250% in hepatic damage from carbon tetrachloride. From the spectrophotometric method in puppies administered 7 mg./kg. of the dye and sample collection at eight minutes, McKibbin et al. (1944) accepted three to eight micrograms per ml. as normal and found values of 12 to 42 micrograms in fatty metamorphosis produced by choline deficiency. Svirbely et al. (1946) by spectrophotometric technique on samples from dogs given 5 mg./kg. of bromsulfalein and sampled at five and thirty minutes, obtained average normal retentions ranging from 2.5 to 13.2% (average 6.2%). In xylidine-damaged livers, they recorded values as high as 65% and in fatty metamorphosis values of 14.1% when the control reading was 4.8%. In his application of liver function tests in dogs, Noyan (1949) appraised the value of the bromsulfalein test by comparing the results in fifty normal dogs with those in dogs with miscellaneous disturbances such as distemper, bile duct ligation, fasting and in dogs receiving pentobarbital

sodium, sulfamerazine, arsenamide sodium, carbon tetrachloride and fox encephalitis virus. The technique entailed injection of 5 mg./kg. of the dye and sampling of the blood in thirty minutes. To each cc. of serum, 4 cc. of distilled water, 1 to 2 drops of 10% NaOH or 1 to 2 drops of 5% HCl (control) were added. Percentage of dye retention was determined in a Rouy-Leitz photometer. Normal dogs were always below 10% retention with the majority at 5%. The highest retentions (64%) resulted from hepatic damage in the form of fatty metamorphosis caused by carbon tetrachloride. In seven of twenty-four dogs afflicted with distemper, retentions up to 10% were recorded and in sera from puppies injected with fox encephalitis virus as high as 27% of the dye was retained. He found false positives were attributable to fever, heart failure, ascites, obstructed bile ducts and parenterally injected dyes. He considered the test more reliable in acute than in chronic liver disorders and valuable in hepatitis and fatty degeneration but questionable in cirrhosis. Hoerlein and Greene, using the method of Rosenthal and White (1925), which utilizes the comparator block, but substituting 5 mg./kg. of the dye instead of the 2 mg./kg. dosage, concluded that no retention at 30 minutes was normal. In fifty clinical cases, twenty-two had abnormal values of 5 to 10% and three showed 100% retention. Among sixteen miscellaneous clinical cases, two of three animals with distemper had 10% retentions. They concluded that the test was of value in confirming early hepatic dysfunction, such as cirrhosis, fatty hepatosis, and other liver conditions in which the animal does not show abnormal bilirubinemia, and that it was quantitative in indicating the amount of liver damage.

Lamson and Wing (1926b) demonstrated that as little as 0.3 cc. of carbon tetrachloride produced early cirrhosis in dogs if given every other day for fifteen weeks. They had previously reported (1926a) that threshold doses of 0.5 to 1.0 cc./kg. produced central necrosis of the liver.

MATERIALS AND METHODS

A. The Bromsulfalein Liver Function Test

The bromsulfalein used in these studies was the commercial* 5% (50 mg./ml.) aqueous solution of Sulfobromophthalein Sodium, U.S.P., (Phenoltetrabromphthalein - disodium sulfonate). For rat use, the commercial preparation was prepared by dilution with sterile isotonic sodium chloride solution to a final concentration of 1 mg./ml. Later in the studies, the concentration was increased to 2 mg./ml. to reduce the volume injected. In the dog, the undiluted commercial solution was used. The dye was administered at the rate of 5 mg./kg. body weight to all animals.

After collection, the blood sample was transferred to a centrifuge tube and allowed to clot. The sample was centrifuged for ten minutes at 2000 r.p.m. for rat blood or 3500 r.p.m. for dog blood. Serum sufficient for the test was transferred to a clean test tube. If the determinations were not to be made promptly, the serum was refrigerated.

The photoelectric colorimeter used in these experiments was a Beckman Model B Spectrophotometer.

Hepler's (1949) procedure, which is essentially that of Gaebler (1945), was followed in the estimation of bromsulfalein retention. The technique of the test is as follows:

1. 0.5 ml. of serum was accurately measured into each of two x 25 mm., flared-lip, thin-walled test tubes.

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* Hynson, Westcott and Dunning, Inc., Baltimore, Maryland.

2. 2.5 ml. of deionized water (distilled water is recommended) was added to each tube.

3. 3.0 ml. of 0.1N NaOH was added to one tube, to develop the color, and mixed.

4. 3.0 ml. of 0.1N HCl was added to the other tube, to serve as a blank, and mixed.

5. The tubes were allowed to stand for five minutes to insure full development of the color.

6. Each of the mixtures was added to the standard curvette and placed in the spectrophotometer. The wave length was set at 580 mu. Using the blank, the galvinometer was set at 100 and the galvinometer reading was then obtained for the unknown.

7. The percentage of dye retention was obtained from a table previously prepared by calibration of a standard curve as described in the reference (Hepler, 1952).

This procedure was followed on the sera from dogs.

Because of the small quantity of blood collected from the rats, the volumes of the sera and reagents were reduced by one-half - 0.25 ml. of serum, 1.25 ml. of deionized water and 1.5 ml. each of 0.1N NaOH and HCl. The total volume was sufficient to fill the aperture in the carrier and not leave a meniscus to distort the transmission of the light beam.

B. Procedures on the Rat

Rats of Wistar strain from the Upjohn colony were used in these studies. Their weights ranged from 129 to 392 grams. They were predominantly males.

At the time of injection of the dye solution, or prior to

heart puncture or surgical manipulation, the rats were anesthetized with cyclopal at 80 mg./kg. given intraperitoneally.

Immediately before the test was to be performed, the rat was weighed, the dose calculated, and the injection made with a 1/2-inch, 27-gauge needle.

Regardless of route of administration of the bromsulfalein solution, blood samples for the determination were collected by cardiac puncture. The hair was clipped over the thoracic area and the puncture made through the thoracic wall with a 1/2-inch, 23-gauge needle with clean, dry syringe attached. Three to four ml. of blood were withdrawn.

The animals were sacrificed within several hours after blood samples were collected if hepatotoxic agents were administered, or as late as 24 hours afterward if they were controls. Euthanasia was accomplished by severing the cervical spinal cord with heavy rib forceps. The viscera were exposed and examined for lesions with particular attention being paid to the liver. The hepatic parenchyma was examined after exposure with multiple incisions.

To obtain average bromsulfalein retention values, the following methods were used.

1. Retention values were secured on eighteen animals, eight males and ten females, ranging in weight from 126 to 190 grams, by injecting the dye solution intraperitoneally and collecting the blood sample in forty-five minutes.

2. Dye retention was determined on fifteen rats, of which twelve were male and three female ranging in weight from 140 to 197 grams, after the solution had been injected into the coccygeal vein;

blood samples were collected at five minutes from one rat, at fifteen minutes from thirteen animals, and at thirty minutes from one.

3. In ten males, with weight range of 299 to 377 grams, the dye solution was injected into the saphenous vein which had been exposed by incising the skin over the vessel; the determinations were made on blood samples collected at thirty minutes.

On rats that sustained damage to the liver by hepatotoxic agents, bromsulfalein retention determinations were obtained utilizing the peritoneal route of injection and forty-five-minute sampling technique.

1. Twelve male rats, 309 to 392 grams in weight, were exposed three times, in a closed container to ten minutes of intermittent chloroform anesthesia with three-hour intervals between exposures. Intermittency was produced by inducing deep narcosis, then allowing partial recovery, followed by deep narcosis with the cycle repeated a number of times during the ten minute periods. Respiratory failure often was corrected by artificial respiration and the animal again submitted to the chloroform vapors during the same period.

2. Larson (1956) demonstrated that pyrazolidin-3-one, 1-methyl-5-phenyl, in rats, induced bile stasis with inspissation and marked proliferation of bile ducts. Twenty-four rats, twelve of each sex, ranging in weight from 136 to 209 grams, were administered 100 mg./kg. of the drug for five days and then 200 mg./kg. for the next three successive days. Retention of dye was determined on eleven males from this group.

Negative findings, with few exceptions, are not recorded in this thesis.

C. Procedures on the Dog

The dogs in these studies ranged from nondescript crossbreds to purebreds and were from a variety of sources. The majority of the beagles were from the Upjohn colony or secured for the colony from local breeders; complete histories of these dogs were available. Dogs of doubtful breeding, recognizable crossbreds and a number of probable purebreds were from dog pounds within limited geographical radius from the laboratory; histories were not obtainable on these.

The animals ranged in age from several months to aged but the majority were one to two years old. Both sexes were represented.

The Upjohn colony dogs were treated for intestinal parasites, inoculated against canine distemper and infectious hepatitis, and given complete physical examinations, including routine clinico-pathological tests, before being assigned to toxicological tests.

The animals were weighed and the dosage calculated immediately before the test was to be performed. The hair was clipped over the radial aspect of the foreleg and the dye solution injected into the cephalic vein using a 1-inch, 21-gauge needle.

The hair was clipped from the ventral aspect of the neck and at 45 minutes after injection a 7 to 8 ml. blood sample was withdrawn from the jugular vein. A 1 1/2-inch, 19-gauge needle with attached clean, dry syringe was used to collect the sample.

The dogs were sacrificed on the same day or not later than the third day following the performance of the function test. All of the animals were killed by electrocution. Partial exsanguination immediately followed.

A general necropsy was performed on each dog but the brain and bone marrow were examined only in the animals on the drug-intoxication experiments. Blocks of tissue were removed, fixed in 10% formalin solution, embedded in paraffin, sectioned at 6 microns, and stained with hematoxylin and eosin. Fat-stained sections, formalin-fixed, were prepared either by the freezing method or embedded in Carbowax and stained with Sudan IV or oil red O. All of the sections were examined microscopically.

The tissues and organs routinely examined histologically varied with the different studies but any grossly pathologic tissue was saved, sectioned, stained and examined. Table 1 sets forth the organs and tissues routinely subjected to microscopic examination.

The bromsulfalein liver function test was performed and necropsy and histopathological examinations completed on eighty-five dogs: fourteen were normal controls; five had received carbon tetrachloride; thirty-five had received other drugs; twenty were exhibiting symptoms of the distemper complex; six had been inoculated with canine infectious hepatitis virus; and five were pyretic only. Of the thirty-five animals which had been administered drugs, eight had received the sodium salt of tolbutamide, nine 3,5 - dipropyl-4-allyloxy-benzoic acid, six pyrazolidin-3-one, 1-methyl-5-phenyl, six the antibiotic streptovaricin, and six methyl reserpate.

The normal dogs were purebred colony beagles with normal physical (two examinations) and clinico-pathologic findings. They had served as controls for toxicopathologic studies and had received only empty gelatin capsules.

Two male and three females of mixed breeding, ranging in weight

from 5.9 to 9.4 kilograms, composed the group which received the carbon tetrachloride. All recorded bromsulfalein retention values of 0.8% on the pretreatment determinations. A regimen of 0.25 ml. of carbon tetrachloride in a gelatin capsule administered daily, seven days a week was instituted initially for all dogs. After eight days, the dosage was increased to 0.4 ml. in Dogs. 6664 and 6796, the former remaining on this dose for 12 more days and the latter for 14 days. The bromsulfalein retention was determined in Dog 6664 the day after the last dose, after which she was sacrificed. The dose was again reduced for Dog 6796 to 0.25 ml. for the next 38 days. The function test was performed and the dog sacrificed on the eleventh day following the last dose. Dogs 7071, 7072, and 7073 received the initial level for 38 days, were untreated for 11 days, and then given 0.5 ml. daily for 79 days. The day following the last dose, the function tests were performed and the animals killed.

Beagles from the colony were employed in all of the drug studies. The number of each sex in the studies varied from drug to drug. For one reason or another, not all the dogs in each test were included in this study. The drugs were administered in gelatin capsules and given in three divided doses daily with one exception. The procedural data for each drug are set forth individually below:

1. Sodium salt of tolbutamide: Administered at the rate of 30, 100, and 160 mg./kg., to three dogs per dosage level for six months. Eight of the nine animals, two males and six females, were included in this evaluation.

2. 3,5-dipropyl-4-allyloxyl-benzoic acid: Administered at

approximately 30, 100, 300 and 525 mg./kg., for one month; three dogs per level for the first three dosage levels and one dog for the highest level were used. Five males and four females constituted the group in this evaluation.

3. Pyrazolidin-3-one, 1-methyl-5-phenyl: Administered at approximately 30, 100/60, and 300/60 mg./kg., three dogs per dosage level, for 28 to 35 days. The 300 mg./kg. dosage was discontinued after five doses and the 100 mg./kg. dosage after 23 doses. The dosage was continued at 60 mg./kg for the remainder of test. One male and five females were included in this evaluation.

4. Streptovaracin: Administered at approximately 30, 100 and 300 mg./kg., to male dogs, three at each level of drug, for 64 days. Six males from this group were included in this evaluation.

5. Methyl reserpate: Administered at approximately 5, 15, and 50 mg./kg., once daily, for 21 days and at approximately 1 mg./kg., once daily, for 28 days. Six (one male and five females) of the seven dogs in this group were evaluated.

Fifteen male dogs, five of which were colony beagles and the remainder of pound origin, and five females, all of pound origin, manifesting symptoms of the canine distemper complex, were subjects of this study. Presumptive diagnosis was made from physical examination.

Eight very immature pound dogs (three males and five females) were inoculated subcutaneously on two successive days with 0.5 ml. of ICHVR (Upjohn) infectious canine hepatitis virus. This virus had proved its pathogenicity in susceptible animals*. Body temper-

* E. A. Slater. The Upjohn Company, Kalamazoo, Michigan: Personal communication.

atures were recorded and the dogs examined daily after the fourth post-injection day. The procedures were accomplished on the twelfth day after inoculation on Dog 6492, the eighth day on Dog 6681, and on the seventeenth day on Dogs 6697, 6760, 6761, and 6762.

Five immature pound dogs (one male and four females) of mixed breeding, secured for the experimental canine infectious hepatitis study, were found on preliminary physical examination to have elevated body temperatures but no other physical signs of disease. The liver function test and euthanasia were performed on the same day as the physical examination.

TABLE 1

ORGANS AND TISSUES EXAMINED MICROSCOPICALLY

A - Distemper Complex Dogs
B - Canine Infectious Hepatitis Dogs
C - Carbon Tetrachloride Dogs
D - Dogs on other Drugs

Organs and Tissues	A	B	C	D
Cerebrum				x
Cerebellum				x
Pituitary				x
Eye				x
Lower tarsal conjunctiva	x			
M. nictitans	x			
Turbinate	x			
Tonsil	x	x	x	x
Salivary gland				x
Thyroid				x
Mandibular lymph node	x	x	x	x
Suprapharyngeal lymph node	x	x	x	x
Lung	x	x	x	x
Trachea (at bifurcation)	x	x		
Heart				x
Aorta				x
Liver (left and right lateral and caudate lobes)	x	x	x	x
Hepatic lymph node			x	
Cholecyst	x	x	x	x
Pancreas	x		x	x
Adrenal				x
Spleen	x	x	x	x
Mesenteric lymph node	x	x	x	x
Stomach	x	x	x	x
Duodenum				x
Jejunum				x
Ileum				x
Cecum				x
Colon				x
Kidney	x	x	x	x
Urocyst	x	x	x	x
Testis	x	x	x	x
Prostate	x	x	x	x
Ovary			x	x
Uterus				x
Skeletal muscle				x
Bone marrow				x

RESULTS

A. Bromsulfalein retention in the rat.

1. In the normal rat. The percentages of retention of the dye in the normal rats under cyclopal anesthesia, when it was injected intraperitoneally and the blood sample collected at forty-five minutes, varied between 0.8 and 2.2% (Table 2). In the males, three retained 0.8%, three 2.2% and one 1.5%. In the females, one retained 1.5% and the nine others, 0.8% each. There was slight hemolysis in the sera from one male and two females.

These rats were sacrificed within two hours after the blood samples were collected. Before the viscera were inspected for lesions, two ml. of 10% sodium hydroxide solution were introduced into the abdominal cavity; the pale violet color of alkaline bromsulfalein developed distinctly in all the animals.

No abnormalities of color or architecture of the liver were detected grossly and lesions in other organs and tissues were not observed in these animals.

The possibility that the dye was not completely absorbed from the peritoneal cavity necessitated studies to determine the clearance of the bromsulfalein from the blood stream when it was injected directly into the vascular system. A pilot study using three rats was conducted and the results suggested that a fifteen minute sample of the blood would be a sensitive indication of clearance of the dye from the blood stream. However, the results (Table 3) from the coccygeal vein injection were erratic, ranging from 0.8 to 6.8% retention. The high incidence of hemolysis of the samples in rats

11 through 06 suggested that the volume of dye solution was causing hemolysis *in vivo*. The concentration of dye was increased to 2 mg./ml. in rats 07 through 012 to reduce the volume to be injected, and evidence of hemolysis was less prominent.

A number of considerations led to the use of the saphenous vein as the route of injection and thirty minutes as the time of collection of the blood sample (see Discussion). Bromsulfalein retention did not exceed 2.2%, the majority retained 0.8% (Table 4).

2. *In rats receiving hepatotoxic agents*. Two rats succumbed during exposure to chloroform and data from them are not recorded. At least six of the animals required artificial respiration during one or more of the periods of narcosis. On the day following, which was the day the function test was performed, all the rats were mildly depressed. Post-mortem examination revealed that five of the eleven animals had demonstrable liver lesions (Table 5) indicative of degeneration of the parenchyma. Ten rats had dye retention values of 0.8% and one retained 1.5%.

The sera of the animals receiving pyrazolidin-3-one, 1-methyl-5-phenyl were intensely icteric but otherwise clear. A retention of 0.8% bromsulfalein (Table 6) was exhibited by ten rats and 3.0% by one rat. Ten of the eleven animals had grossly visible defects in the liver (Table 6). The lesions were similar to those observed in rats on a subacute toxicity test which included gross and microscopic examinations of the liver. Continuation of the procedures in the females was considered useless in view of the results obtained from the males.

TABLE 2

CONTROL RATS

45 MINUTE SAMPLE

Rat No.	Weight (gms.)	Sex	% Retention	
7	190	M	0.8	1+
8	166	M	2.2	1+
9	172	M	0.8	
11	177	M	2.2	1+
12	180	M	2.2	
13	129	M	0.8	
14	141	M	0.8	
16	143	M	1.5	+
20	133	F	1.5	1+
21	138	F	0.8	+
22	135	F	0.8	+
23	141	F	0.8	
24	126	F	0.8	
25	165	F	0.8	
26	140	F	0.8	
28	159	F	0.8	
29	147	F	0.8	
30	147	F	0.8	

+ = trace of hemolysis

1+ = slight hemolysis

TABLE 3

CONTROL RATS

COCCYGEAL VEIN

Rat No.	Weight (gms.)	Sex	Sample/Minutes	% Retention	
1i	166	F	5	5.1	1+
2i	171	F	15	0.8	+
3i	197	F	30	4.3	1+
01	158	M	15	1.5	
02	165	M	15	3.0	1+
03	140	M	15	1.5	1+
04	141	M	15	0.8	
05	160	M	15	6.8	1+
06	143	M	15	0.8	1+
07	169	M	15	2 2	
08	163	M	15	0.8	+
09	172	M	15	0.8	
010	146	M	15	0.8	
011	168	M	15	3.6	+
012	150	M	15	0.8	+

+ = trace of hemolysis

1+ = slight hemolysis

TABLE 4

SAPHENOUS VEIN

30 MINUTE SAMPLE

Rat No.	Weight (gms.)	Sex	% Retention
B1	356	M	0.8
B2	370	M	1.5
B5	299	M	0.8
B6	336	M	0.8
B7	377	M	2.2
B8	309	M	0.8
B9	346	M	0.8
B10	346	M	0.8
B11	321	M	1.5
B12	303	M	0.8

TABLE 5

CHLOROFORM INTOXICATION

45 MINUTE SAMPLE

Rat No.	Weight (gms.)	Sex	% Retention		Gross Appearance of Livers
55	316	M	1.5	1+	Essentially normal.
57	309	M	0.8		Faint yellow cast.
58	392	M	0.8		Slight yellowish mottling.
59	342	M	0.8	+	Slightly swollen but normal in color.
60	365	M	0.8		Essentially normal.
61	364	M	0.8		Essentially normal.
62	326	M	0.8		Essentially normal.
63	377	M	0.8		Essentially normal.
64	366	M	0.8	1+	Slight yellowish mottling.
65	387	M	0.8		Essentially normal.
66	315	M	0.8		Slight yellowish mottling.

+ = trace of hemolysis

1+ = slight hemolysis

TABLE 6

PYRAZOLIDIN-3-ONE, 1-METHYL-5-PHENYL RATS

45 MINUTE SAMPLE

Rat No.	Weight (gms)	Sex	% Retention*	Gross Appearance of Livers
31	201	M	0.8	Essentially normal.
32	191	M	0.8	Yellowish tan in color; central veins or triads very conspicuous.
33	224	M	0.8	Reddish tan in color with dark tan mottling throughout; loss of architecture.
34	190	M	0.8	Left lateral lobe tan in color; median lobe reddish tan with circumscribed whitish areas near posterior border; right lobe reddish tan with multiple whittish areas; caudate lobe tan in color.
35	177	M	0.8	Major parts of left lateral and median lobes tan with red mottling - remainder of lobes normal.
36	139	M	3.0	Light tan diffuse areas disseminated throughout.
37	203	M	0.8	Normal in color but lobulation indistinct.
38	209	M	0.8	Multiple, tan areas throughout.
39	226	M	0.8	Multiple, tan areas throughout.
40	187	M	0.8	All lobes tan in color with loss of lobular architecture except in several areas which were normal structurally but pale pink in color.
42	181	M	0.8	Dark reddish tan areas with loss of lobular architecture interspersed with lighter tan fields with normal structure.

* - all sera icteric.

B. Bromsulfalein retention in the dog.

1. In healthy, untreated dogs. Dye retention of 0.8% was recorded for thirteen and 1.5% for one of the fourteen control dogs which were free from clinical signs of disease and were not recipients of drugs or infectious agents. On necropsy, however, eight of the fourteen had grossly discernible lesions in various organs and tissues. The livers, both grossly and microscopically, were lesionless. The histopathological examination of various other organs revealed a wide variety of spontaneous lesions. The results of the liver function test and the gross and microscopic examinations are in detail in Appendix 1.

2. In dogs administered carbon tetrachloride. All the dogs receiving this agent lost over a kilogram of initial body weight during the test period. Dog 6664 was killed after 21 doses of the chemical because severe stomatitis developed. Marked jaundice was present in Dog 6796 after 10 days on the 0.4 ml. regimen and 5 days later the dose was reduced. Body weight loss, poor condition, and, later, slight loss of appetite were the only physical manifestations displayed by Dogs 7071, 7072, and 7073. Dog 6796 was kept for 11 days following the last administration of carbon tetrachloride to allow the acute phase of the intoxication to subside before authanasia.

Bromsulfalein retention ranged from 0.8 to 34.0% in the carbon-tetrachloride-intoxicated dogs (Appendix 2). Dog. 6796, with a dye retention of 0.8%, had residual hepatic damage consisting of hepatocellular swelling, mild cirrhotic change and centrolobular bile stasis. Severe hepatocellular degeneration and disturbed circulation were primary histologic alterations in the liver of Dog 6664 which had a

21.8% retention. In Dogs 7071, 7072, and 7073, the dye retention of 34.0, 29.8 and 23.6%, respectively, varied inversely to the degree of cirrhosis encountered and directly with acute damage in their livers.

3. In dogs receiving drugs. The results of the liver function test and the gross and microscopic examinations are given in detail in Appendix 3. To simplify the discussion of findings, both under this heading and Discussion, the drugs will be assigned an alphabetical designation:

Drug A	Streptovaricin
Drug B	Tolbutamide, sodium salt
Drug C	Dipropyl-allyloxy-benzoic acid
Drug D	Pyrazolidin-3-one, 1-methyl-5-phenyl
Drug E	Methyl reserpate

Bromsulfalein retentions ranged between 1.5 and 9.0% in dogs receiving Drug A. At 30 mg./kg., retentions in Dogs 5924, 5925, and 5926 were 2.2, 2.2, and 4.8%, respectively, but the gross and histologic examinations of the livers disclosed no abnormalities. In the dogs receiving 100 mg./kg., Dog 5927, with 3.6% retention, had no gross or microscopic hepatic lesions. Dog 5928 retained 1.5% of dye in the serum and showed focal lymphocytic infiltration or proliferation in the hepatic parenchyma. With a 9.0% retention, Dog 5929 showed an early state of an acute purulent hepatitis microscopically.

At 30 mg./kg. of Drug B, the retentions in the three dogs were 0.8, 0.8, and 1.5% without hepatic alteration. At 100 mg./kg. of the drug the dye retention was 2.2% (Dog 6078) and 1.8% (Dog 6080),

without discernible liver damage, and 4.3% in Dog 6079 which had diffuse hepatocellular vacuolization. In the two dogs (6081 and 6082) administered 160 mg./kg. of Drug B, 4.3 and 1.5% values for the function tests were recorded; mild diffuse subacute hepatitis was detected microscopically in the livers of both dogs. Gross pathologic hepatic change was seen in Dog 6081 but not in Dog 6082.

The livers were essentially normal macroscopically and microscopically in the subjects on 30 mg./kg. of Drug C; dye retentions were 2.2, 0.8, and 0.8%. Retentions were 0.8% in each of the three dogs on 100 mg./kg. of the drug. Dog 6194, however, displayed an allergic hepatic response, histologically, characteristic of parasitic injury; the livers in the other two dogs were essentially normal. One dog (6198), at 300 mg./kg., retained a serum concentration of dye of 41.2% and showed hepatic congestion and degeneration of hepatic cells at necropsy and microscopically. There was no apparent hepatopathy in the other two dogs and retention of bromsulfalein in both dogs was 0.8%.

Hepatopathy of varying degrees occurred in all the dogs included in the study from the Drug D evaluation. Likewise, elevated dye retentions were recorded for all the subjects. Hepatotoxic manifestations observed at necropsy consisted principally of alterations in the color of the organ and less consistently in changes in texture. Histologically, the lesions were characterized by pigment (probably hematogenous) accumulation and damage to the hepatic cells and vascular bed. The intensity of these changes paralleled somewhat the level of the drug and was reflected, generally, in the concentration of bromsulfalein in the sera. In Dog 6247, with 6.2% retention,

the tissue alterations were relatively mild whereas in Dog 6243 the hepatic damage was marked, the pigment abundant and blood clearance of the dye was delayed, resulting in a 21.8% retention. Extending the sampling of the blood to an hour and forty-five minutes resulted in a 7.0% retention at that time in Dog. 6241 which sustained severe hepatic damage from the drug.

Bromsulfalein retentions of 0.8% were found in the six subjects receiving Drug E. With one exception, the livers of these dogs were free of abnormal change. Microscopic examination of the liver of Dog. 6470, on 1 mg./kg. of the drug, disclosed a very mild, focal, subacute hepatitis.

4. In dogs affected by infectious disease.

a. In dogs with clinical signs of the distemper complex. The results of the liver function tests, and the clinical, post-mortem, and histopathological examinations are in detail in Appendix 4.

A definitive diagnosis of canine distemper based on the presence of intranuclear or intracytoplasmic inclusion bodies in tissues was made in six of the twenty dogs (6337-4, 6337-6, 6337-9, 6337-10, 6338-c, and 6443-6).

Eight dogs had bromsulfalein retentions of 0.8%; retentions of 1.5 to 9.8% were recorded for the other twelve dogs. In the former, one liver showed early degenerative changes and one contained lesions of mild focal subacute hepatitis. The livers of the subjects having retentions 4.0% and over contained, principally, lesions indicative of focal hepatitis, centrolobular necrosis or centrolobular hemorrhage and congestion. In the cases of retentions below 4.0%, degenerative

changes predominated in the livers but there was one case of mild focal hepatitis and one of larval granulomatosis which was identified by its similarity to larvae-containing lesions observed in dogs in previous studies at this laboratory. Both animals had a 2.2% of dye.

b. In dogs injected with infectious canine hepatitis virus. In the first series, three dogs were recipients of the virus. Two of the three developed persistent low-grade fevers beginning on the fifth day and subsiding on the eleventh day after injection. The other puppy was afebrile for six days after injection, then sustained a rise in body temperature to 104.6°F. with serous ocular and nasal discharges and died unexpectedly two days later. The function test was executed in the two remaining dogs on the eleventh day after injection. The dye retentions were 0.8% and 5.9%, respectively. Only the dog with the elevated retention was evaluated pathologically (Appendix 5). Evidence of a systemic infection was apparent grossly and microscopically in this animal but the typical hepatic intranuclear inclusion bodies were not evident. The liver sections were characterized by mild focal subacute hepatitis, moderate centrolobular fatty metamorphosis, and inexplicable tinctorial nuclear changes which were chacterized by reversal of staining affinity or mauve coloration of the nucleoli.

Febricity was not a characteristic sign in the second series of dogs receiving the virus. All the subjects, however, lost condition repidly after the seventh post-injection day. Dog 6681 was in such debilitated condition, but with no other discernible signs, as to require sacrifice, after the function test was performed, on the eighth post-injection day. Debility was the only clinical sign of

disease evident in Dogs 6760, 6761, and 6762 during the observation period but Dog 6697 was emaciated, weak, and presented an ocular and nasal discharge, terminally, on the seventeenth day.

Dog 6681 had a 4.3% retention of dye. Grossly the liver had a yellowish cast and there was a very mild acute focal hepatitis histopathologically. Dogs 6760, 6761, and 6762 had shown dye retentions of 0.8%. The livers were not abnormal grossly and, in Dogs 6760 and 6761, the organ was essentially normal microscopically. Moderate focal, subacute (bordering on chronic) hepatitis was detected in Dog 6762. The hepatic lesions in Dog 6697 were characterized macroscopically by irregular yellowish mottling and microscopically by disseminated acute focal hepatitis; bromsulfalein retention was 12.0%.

Intranuclear inclusion bodies were not engendered by this virus.

5. In dogs with pyrexia. The bromsulfalein retention in each of the five dogs was 0.8% (Appendix 6). The livers were essentially normal on macroscopic and microscopic examinations, with two exceptions: Dog 6446-1 had mild hepatic larval granulomatosis and Dog 6446-5 had very mild hepatocellular cloudy swelling. In the former, the lesions were focal, confined and two in number. The cause of fever could be determined with certainty in only two of the animals. Dog 6446-1 was in the early stages of bronchopneumonia and Dog 6446-2 had severe unilateral tonsillitis and lymphadenitis of the suprapharyngeal lymph node. The lesions (hemorrhage, lymphocytic depletions, and/or lymphoid hyperplasia) in several of the lymphoid tissues detected grossly or in the tissue sections from Dogs 6446-2, 6446-3, 6446-4, and 6446-5 suggest that they were in

the prodromic stage of the systemic infection, conceivable viral.

Seventy-two of the eighty-five dogs exhibited lesions in one or more organs or tissues, other than the liver and major reticulo-endothelial organs (spleen and lymph nodes). Sixty-one animals had involvement of one or more of the major reticulo-endothelial organs. Lesions were found in other than hepatic loci in animals which had minimal levels of retained bromsulfalein in their serums and in those which retained a high percentage of dye in the blood stream.

A summary of the percentage of retained dye and the histopathological alterations are set forth in Table 7.

TABLE 7

SUMMARY OF HEPATIC HISTOPATHOLOGICAL FINDINGS

Key to agents: N - control; CT - carbon tetrachloride; A,B,C,D - Drugs A,B,C,D; CDC - canine distemper complex; CIH - canine infectious hepatitis virus; P - pyrexia.

Dog No.	Sex	Wt. (Kg.)	Agent	BSP ret.	Gross evidence		Hepatic histopathology
5921	M	7.9	N	0.8	-	-	
5922	M	7.7	N	0.8	-	-	
5923	N	8.4	N	0.8	-	-	
6072	M	6.6	N	0.8	-	-	
6073	F	6.5	N	0.8	-	-	
6074	F	6.1	N	0.8	-	-	
6188	M	10.8	N	0.8	-	-	
6189	M	11.9	N	0.8	-	-	
6190	F	9.6	N	0.8	-	-	
6249	F	12.0	N	0.8	-	-	
6250	F	11.2	N	1.5	-	-	

6251	M	8.5	N	0.8	-	-
6468	M	9.8	N	0.8	-	-
6469	M	12.5	N	0.8	-	-
6664	F	4.3	CT	21.8	X	Centrolobular degeneration; infrequent necrosis; severe centrolobular fatty metamorphosis.
6796	M	7.2	CT	0.8	-	Hepatocellular swelling; mild fibrosis; bile stasis.
7071	M	7.8	CT	34.0	X	Severe degeneration; hepatocellular necrosis; hemorrhage; mild perilobular fibrosis; mild bile duct proliferation; mild regeneration.
7072	F	7.0	CT	29.3	X	Severe degeneration; centrolobular hemorrhage; pigment accumulation; mild perilobular and periportal fibrosis.
7073	F	6.1	CT	23.6	X	Hepatocellular degeneration and regeneration; moderate peripherolobular fibrosis and hemorrhage; mild bile duct proliferation and pigment accumulation.
5924	M	9.2	A	2.2	-	-
5925	M	9.5	A	2.2	-	-
5926	M	8.9	A	4.8	-	-
5927	M	7.8	A	3.6	-	-
5928	M	8.3	A	1.5	-	Scattered foci of lymphocytes.
5929	M	8.2	A	9.0	-	Central vein perivasculitis.

6075	M	8.4	B	0.8	X	- (*)
6076	M	7.8	B	0.8	X	- (*)
6077	F	8.0	B	1.5	-	-
6078	M	9.0	B	2.2	-	-
6079	F	8.1	B	4.3	-	Hepatocellular vacuolization (not fat).
6080	F	7.2	B	1.8	-	-
6081	F	7.9	B	4.3	X	Moderate subacute hepatitis; peripherolobular fatty metamorphosis.
6082	F	8.0	B	1.5	-	Essentially similar to Dog 6081.
6191	F	8.0	C	2.2	-	Scattered inflammatory foci and pigment.
6192	M	9.5	C	0.8	-	-
6193	M	9.2	C	0.8	-	-
6194	F	7.6	C	0 8	-	Focal allergic reaction (parasitic).
6195	F	8.9	C	0.8	-	-
6196	M	7.1	C	0.8	-	-
6197	M	9.4	C	0.8	-	-
6198	F	6.4	C	41.2	X	Centrolobular congestion and hepatocellular degeneration
6199	M	8.6	C	0.8	-	-

6246	F	7.2	D	9.8	X	Moderate centrolobular accumulation of pigment; centrolobular endothelial hypertrophy and mild hemorrhage; mild fatty metamorphosis.
6247	F	7.2	D	6.2	X	Mild centrolobular accumulation of pigment and fatty metamorphosis; bile duct epithelial hypertrophy.
6243	M	7.0	D	21.8	X	Centrolobular: hepatocellular degeneration and necrosis, pigment accumulation, stromal degeneration and marked fatty metamorphosis.
6244	F	7.9	D	10.5	X	Centrolobular: pigment accumulation, marked hepatocellular degeneration, mild fatty metamorphosis.
6241	F	7.5	D	7.0	X	Centrolobular: moderate pigment accumulation, marked hepatocellular degeneration and occasional necrotic foci generalized moderate fatty metamorphosis.
6470	F	7.4	E	0.8	-	Very mild focal subacute hepatitis.
6471	F	12.4	E	0.8	-	-
6463	F	9.8	E	0.8	-	-
6464	F	8.8	E	0.8	-	-
6465	M	7.0	E	0.8	-	-
6466	F	6.5	E	0.8	-	-
6534-1	F	7.0	CDC	0.8	-	Very mild, early degenerative changes.
6534-2	M	22.0	CDC	2.5	X	Mild hepatocellular degeneration.

6334-3	M	7.0	CDC	4.8	X	Hepatocellular degeneration and centrolobular congestion.
6334-543	M	11.6	CDC	0.8	-	Mild hepatocellular degeneration.
6337-4	M	9.3	CDC	0.8	X	Mild passive congestion and hepatocellular degeneration.
6337-5	M	6.4	CDC	1.2	-	Mild hepatocellular degeneration.
6337-6	F	7.5	CDC	0.8	-	-
6337-7	M	8.8	CDC	0.8	X	Mild to moderate, focal, acute to subacute hepatitis.
6337-8	F	7.0	CDC	6.2	-	Generalized hepatocellular degeneration.
6337-9	F	5.7	CDC	9.8	X	Marked focal acute hepatitis.
6337-10	M	7.6	CDC	5.9	X	Moderate vascular degeneration; moderate focal subacute to chronic hepatitis; larval granulomatosis.
6338-a	M	9.4	CDC	2.2	-	Mild focal, acute to subacute, hepatitis.
6338-b	M	10.3	CDC	0.8	X	Very mild focal subacute hepatitis.
6338-c	M	10.0	CDC	1.5	-	Very mild focal subacute hepatitis.
6344-1	F	7.2	CDC	1.8	X	- (*)
6344-2	M	11.3	CDC	0.8	-	-
6345-3	M	10.0	CDC	4.0	-	Moderate focal acute hepatitis.
6345-4	M	9.3	CDC	1.5	-	Very mild hepatocellular degeneration.
6345-5	M	7.8	CDC	0.8	-	-

6443-6	M	8.2	CDC	2.2	X	Larval granulomatosis.
6492	F	6.9	CIH	5.9	X	Moderate hepatocellular degeneration; mild focal subacute hepatitis; nucleolar polychromatism.
6681	M	7.5	CIH	4.3	X	Very mild acute hepatitis; diffuse hepatocellular degeneration.
6760	F	5.2	CIH	0.8	X	- (*)
6761	M	10.8	CIH	0.8	-	-
6762	M	9.6	CIH	0.8	-	Moderate focal, subacute to chronic, hepatitis.
6697	F	4.4	CIH	12.0	X	Marked focal acute hepatitis.
6446-1	F	7.2	P	0.8	-	Larval granulomatosis.
6446-2	F	8.7	P	0.8	-	-
6446-3	F	5.2	P	0.8	-	-
6446-4	F	6.5	P	0.8	-	-
6446-5	M	8.9	P	0.8	-	-

(*) Histopathological examination did not confirm gross observations.

DISCUSSION

The application of the BSP test to normal rats revealed a number of factors that require clarification. The indication that at least part of the bromsulfalein injected into the peritoneal cavity was still present therein places a questionable value on the results obtained from the forty-five-minute blood sampling. If the amount of dye in the abdominal cavity was within narrow limits in all animals the measure of blood clearance would be more valid. This could be readily ascertained if it were the only problem inherent in the application of the test to rats. Intravenous injection of the dye solution via the coccygeal vein gave such erratic results in retention that, under conditions of these experiments, this injection site should not be considered. The cause of these results is only speculative. While the saphenous vein route with thirty-minute serum sampling, gave the most uniform results in the limited number of animals, the requisite surgical manipulation precludes this method from routine use in a large number of animals. However, the percentages of dye retention found in these animals and those injected intraperitoneally correlated rather closely.

The lack of increased dye retention, even slight, in mild and severe grossly detectable hepatopathy indicates that the method, under conditions of this experiment, was unreliable and would be of questionable value as a routine procedure in rats.

A major objective of this study in dogs included the production and detection of a variety of hepatopathies. Acute to chronic processes were produced. Inflammatory, degenerative, necrotic, vascular, and pigment metabolic changes, sometimes occurring as relatively pure entities but more often in combination, were observed. Hepatitis as acute, subacute or, infrequently, chronic, and very mild to marked in intensity and extensiveness affected seventeen subjects. Hepatocellular degeneration, usually centrolobular, was a frequent histologic finding and it was generally of chemical etiology. Congestion, hemorrhage and other vascular alterations were found only in combination with other lesions. This was also true of pigment accumulation, necrosis, and fatty metamorphosis. Lacking from this evaluation are results of the function test in passive congestion, massive necrosis, either infractive or chemically induced, or fatty metamorphosis uncomplicated by other processes.

Although a definitive diagnosis of canine distemper was possible in only six of the twenty dogs diagnosed clinically as such, this does not necessarily negate the clinical definition of the majority of the sick animals. Unfortunately, inclusion bodies are not present in every case of distemper. There was hepatic involvement in three of these animals strongly suggestive of infectious canine hepatitis but, again, typical inclusion bodies were not evident.

Experimental infection with infectious canine hepatitis virus is difficult even under the most ideal conditions. All eight dogs injected with the virus demonstrated some type of reaction indicating their susceptibility. Only two dogs had liver lesions that

approached the classical description but no inclusion bodies were detected. The remaining dogs were infected as evidenced by the clinical observations, and gross and microscopic examinations but did not show typical hepatic damage or inclusion bodies. Dog 6697 was also afflicted with distemper. It is possible that handling the virus reduced its pathogenicity and/or the susceptibility of the test subjects was reduced by previous exposure or inherent resistance. The route of administration, likewise, may have affected the pathogenesis.

All previous work with the BSP function test using photoelectric methods was based on thirty-minute blood sampling and, therefore, direct comparison between the results and those reported previously is not possible. If the findings obtained from several of the dogs administered drugs A and B are eliminated, tentative ranges of retention emerge and allow speculative normal and abnormal limits for interpretation. Under conditions of this experiment, the upper limit of retention in apparently normal Beagle dogs, without hepatopathy, was 1.5% and it can be considered the upper normal limit. Retentions between 1.5 and 4.0% should be considered in the range of suspicion or indicative of at least functional, if not mild cytological, alteration in the liver. Retentions above 4.0% are indicative of definite hepatic dysfunction in which cytological evidence can be expected.

Table 8 points up the fallibility of the function test in the lower retention bracket, i.e., below five percent. Whereas dogs normal by both clinical and necropsy standards did not retain serum dye above 1.5% (and this in one animal only, the majority having

0.8%), fifteen animals presenting mild to moderate hepatic alterations histopathologically retained 1.5% or less. In a percentage evaluation, of fifty-two dogs retaining 1.5% or less of bromsulfalein, 29% demonstrated very mild to moderate microscopic hepatic lesions. Three, or 33%, of nine dogs having retentions between 1.8 and 2.5% had mild lesions. Of the five animals with serum dye values between 3.6 and 4.3%, four, or 80%, had mild to moderate cytologic changes in the liver. In the total evaluation, sixty-eight dogs in this study had bromsulfalein retentions of less than 5.0% and of these, twenty-three, or 34%, sustained very mild to moderate hepatic alterations. The remaining seventeen subjects had retentions over 5.0% and all, or 100%, had moderately to markedly affected livers. Thus, the correlation between bromsulfalein retention and hepatic defects was inconsistent in these studies.

In carbon tetrachloride-intoxicated dogs, in which uniform correlation could be expected, there were inconsistencies between dye retention and intensity of the morbid hepatic state. Dog 6796, although sustaining a moderate degree of hepatic damage, had dye retention in the normal range. The high retentions from the four other dogs was a more accurate indication of the acute cytologic alteration than a measure of the developing chronic reaction because the dog with the most advanced cirrhotic state had the lowest retention.

Dogs receiving Drug A (Streptovaracin) manifested notable difference between retention and the cytologic condition of the liver. Retention was uniformly slightly elevated and lesions were

evident in the liver of only two of the six dogs. Retention of 1.5% in Dog 5928 was not inconsistent with the very mild focal subacute hepatitis but the moderate perivasculitis in Dog 5929 hardly justified the 9.0% dye retention recorded for him. Nor was there cytological evidence for the slight retentions in Dogs 5924, 5925, 5926, and 5927. It is conceivable that this drug, which is an antibiotic, functionally altered the excretory activity of the liver without reflecting visible cellular change. This would further verify the conclusion of previous investigators as to the sensitivity of this test in detecting even subcytological differences in hepatic function.

Five of the eight dogs receiving Drug B (Tolbutamide, sodium salt) were free of microscopic hepatic lesions and the degree of retention in all the animals varied with the dosage level. Dye retentions were not excessive. However, at 160 mg./kg., two dogs which demonstrated hepatic lesions very similar in extent and intensity showed a rather wide variation in the dye retained; one was within the normal limits and the other had a value somewhat compatible with the injury.

In the Drug C (Dipropyl-allyloxy-benzoic acid) group of animals there was good correlation of the two evaluation factors with one exception. Retentions were within normal range and the livers were not abnormal but in Dog 6198 the retention of dye was 41.2%, the highest recorded for any animal in the study. The hepatic lesions would not appear to justify the high retention and it was more likely the result of the severe generalized circulatory collapse.

There was a more reciprocal agreement between retention and

liver injury in the dogs administered Drugs D and E. Definite excretory failure was evident in the livers of dogs receiving Drug D (Pyrozolidin-3-one, 1-methyl-5-phenyl) but lowered dye clearances occurred in those animals sustaining greater hepatocellular alteration in the form of degeneration and necrosis. Conversely, there was no evidence of hepatopathy in the dogs receiving Drug E (Methyl Reserpate) and their test values were within the normal range with the one exception of Dog 6470 which had a mild focal hepatitis but an 0.8% dye retention.

In the distemper complex dogs, correlation of test and hepatopathy was poorest at the border line of retention. Mild to moderate lesions without elevated retention were common in these dogs. Likewise, the type and, to a degree, the extent of hepatic lesions were not reflected in the clearance of dye from the blood stream in several of the animals. Dog 6337-7 had focal hepatitis which contained necrotic foci yet had 0.8% retention whereas Dog 6345-3 had similar defects but a 4.0% retention. In other dogs, however, there was reasonably good correlation between retention and either the intensity of the liver damage or the extensiveness.

Only one dog injected with infectious canine hepatitis virus, and with notable hepatic defects, recorded a false negative function test; in the others, lesions correlated with retention.

Fever, per se, in the dogs tested did not cause elevated retention. These findings are not in agreement with Noyan's (1949).

There was no definite evidence that lesions in other organs, and especially the primarily reticulo-endothelial tissues, caused increased retention of bromsulfalein. A number of dogs had from

infrequent to frequent involvement of other organs and tissues with no hepatopathy and with normal retention. However, in those dogs with marked liver lesions, high retention, and nonhepatic lesions this fact is less evident and there is no irrefutable evidence that at least part of the retention was not caused by lesions in the nonhepatic tissues.

When all facets of this study in dogs are considered, these facts pertaining to the function test emerge: (1) Correlation of dye retention and hepatic lesions was not consistent when both were mild. (2) Dye retentions below 5.0% were least correlative with the state of the liver; elevated bromsulfalein levels without detectable parenchymatous alterations were most commonly encountered (false positive) but the reverse also occurred (false negative). This was particularly true in those cases where evaluation was made in dogs either receiving drugs or afflicted with infectious diseases. (3) The test did not reflect the circumscribed granulomas of parasitic origin or allergic (eosinophilic) reaction in the liver. (4) Correlation between more severely affected livers and reduced blood clearance of the dye was only partially reciprocal and was not uniformily quantitative as suggested by Gornall and Bardawell (1952) and Hoerlein and Greene (1950). The results in this study essentially confirm Noyan's (1949) conclusions that (5) the bromsulfalein function test is most reliable and valuable in acute hepatopathies. (6) The test would appear to be of little value in the differential diagnosis of canine distemper and infectious hepatitis because secondary toxic hepatitis apparently is a common finding in dogs affected with canine distemper. (7) In drug toxico-

pathology the test cannot stand alone as indicative of liver injury although retention of dye suggests alteration of hepatic function.

This evaluation of the BSP function test in the dog was possibly too critical and the histopathological findings, divorced from appraisal of the total clinical and clincopathological evidence, places its usefulness in a questionable position among the clinical laboratory procedures. It merely re-emphasizes the common fallibility of a single biological test in appraisal of a complex pathological state.

TABLE 8

SUMMARY

% retention	Lesions	No lesions	Total
0.8	10	35	45
1.2	1	0	1
1.5	4	2	6
1.8	0	2	2
2.2	3	3	6
2.5	0	1	1
3.6	0	1	1
4.0	1	0	1
4.3	3	0	3
4.8	1	1	2
5.9	2	0	2
6.2	2	0	2
7.0	1	0	1
9.0	1	0	1
9.8	2	0	2
10.5	1	0	1
12.0	1	0	1
16.2	1	0	1
21.8	2	0	2
23.6	1	0	1
29.8	1	0	1
34.0	1	0	1
41.2	1	0	1

SUMMARY AND CONCLUSIONS

1. The bromsulfalein liver function test, following the procedure of Hepler, was performed in sixty-five rats and eighty-five dogs; the competencies of the livers were appraised by necropsy and histopathologic examination.
2. In the normal and hepatically altered rat, in spite of variation in procedure, the test was unreliable under the conditions of the experiment.
3. Dye retention was determined and pathologic evaluation made in: normal dogs; dogs administered carbon tetrachloride; dogs receiving other drugs; dogs given canine infectious hepatitis virus; dogs exhibiting clinical evidence of distemper complex; and dogs with fever but without other readily detectable signs of disease.
4. Under conditions of the study, the normal limit of retention was 1.5% or under; retentions between 1.5 and 4.0% were considered suspicious and above 4.0% as frankly abnormal.
5. Reciprocal agreement between retained dye and cytological hepatic alteration was inconsistent, especially at the lower range of retention. Twenty-nine percent of the dogs with 1.5% retention or less had detectable liver defects of varying degree. The two factors were generally but not uniformily correlative in the higher or abnormal range.
6. The test was most sensitive and correlative in acute hepatopathy.
7. When the test is used in toxicopathological evaluation of drugs, the results should be interpreted with caution.
8. Excessively high dye retention, without correlative liver damage,

occurred in cardiovascular collapse.

9. Pathological states in other organs and tissues, including reticulo-endothelial, appeared inconsequential in relation to bromsulfalein retention.

10. Pyrexia, per se, apparently was not a factor in reduced clearance of dye from the blood stream in this series.

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APPENDIX 1

CONTROL DOGS - BEAGLES

DOG 5921

Male BSP retention: 0.8%

Gross Pathology:

Consolidation of antero-dorsal aspect of right diaphragmatic lobe of lung; hemorrhage in left suprapharygneal lymph node; moderate trichuriasis.

Histopathology:

Liver - essentially normal.

Lung - organization of bronchopneumonia.

Prostate - three large retention cysts and mild hyperplasia.

Suprapharyngeal lymph node - congested.

DOG 5922

Male BSP retention: 0.8%

Gross Pathology:

No gross lesions.

Histopathology:

Liver - essentially normal.

Prostate - subacute interstitial prostatitis.

DOG 5923

Male BSP retention: 0.8%

Gross Pathology:

Focal pneumonia, right diaphragmatic lobe of lung; scar, .5 cm. in diameter, dorsum of right apical lobe of lung.

Histopathology:

Liver - essentially normal.

Lung - subacute focal suppurative bronchopneumonia and pleuritis; circumscribed areas of squamous metaplasia, with fibrous stroma, assuming glandular form and having polymorphic cells in some areas but generally differentiated and exhibiting intercellular bridges.

Mesenteric lymph node - medulla congested.

DOG 6072

Male BSP retention: 0.8%

Gross Pathology:

No gross lesions.

Histopathology:

Liver - essentially normal.

Prostate - cystic dilatation of acini in one lobule, remainder of gland in early stage of hyperplasia.

DOG 6073

Female BSP retention: 0.8%

Gross Pathology:

Mild inflammation of vagina.

Histopathology:

All tissues essentially normal - section from vagina not examined.

DOG 6074

Female BSP retention: 0.8%

Gross Pathology:

Two slightly raised, white, subcapsular foci, 1.5 mm. and 1 mm. in diameter, in the kidney.

Histopathology:

Liver - essentially normal.

Kidney - foci of lymphocytes, plasma cells and numerous eosinophiles in the cortex adjacent to the capsule; focal area of interstitial infiltration with lymphocytes.

Pituitary - one large and many small cysts in pars nervosa.

DOG 6188

Male BSP retention: 0.8%

Gross Pathology:

White, firm mass, 3 cm. in diameter, on posterior border, upper 1/3 of spleen.

Histopathology:

Liver - essentially normal.

Tonsil - mild acute tonsillitis.

Prostate - focal subacute prostatitis.

Spleen - lymphocytoma.

DOG 6189

Male BSP retention: 0.8%

Gross Pathology:

No gross lesions.

Histopathology:

Liver - essentially normal.

Submandibular lymph node - mild hemorrhage in medulla.

DOG 6190

Female BSP retention: 0.8%

Gross Pathology:

No gross lesions.

Histopathology:

Liver - essentially normal.

Kidney - five circumscribed, spherical foci, randomly distributed in cortex, ranging from acute to chronic reaction, resulting from larval migration - remnants of a larva in one focus.

Pancreas - small focus of densely packed eosinophiles, lymphocytes, fibroblasts and a few macrophages with a surrounding zone of proliferating capillaries in the peripancreatic connective tissue.

DOG 6249

Female BSP retention: 0.8%

Gross Pathology:

Marginal fibrosis and numerous small red infarcts, 2-3 mm. in diameter, irregularly scattered along the posterior border of spleen.

Histopathology:

Liver - essentially normal.

Spleen - hemorrhages in some Malpighian corpuscles.

Kidney - larval granulomas.

DOG 6250

Female BSP retention: 1.5%

Gross Pathology:

No gross lesions.

Histopathology:

All tissues essentially normal.

DOG 6251

Male BSP retention: 0.8%

Gross Pathology:

Slight petechiation of mucosa of urocyst; marginal red infarcts in spleen; mild roundworm infection.

Histopathology:

Liver - essentially normal.

Spleen - small hemorrhages in several of the Malpighian corpuscles.

Prepuce - mild follicular balanoposthitis.

DOG 6468

Male BSP retention: 0.8%

Gross Pathology:

Liver dark red in color; two small uroliths in urocyst; area of subperitoneal edema, 7.5 cm. long, in lumbar region just posterior to right kidney.

Histopathology:

Liver - essentially normal.

Spleen - capsular siderosis; mild lymphocytic hypoplasia.

Kidney - infrequent sudanophilic inclusions in glomeruli; fat in convoluted tubules in several areas.

DOG 6469

Male BSP retention: 0.8%

Gross Pathology:

No gross lesions.

Histopathology:

Liver - essentially normal.

Lymph nodes - Section 1: acute eosinophilic lymphadenitis; Section 2: moderate infiltration of medulla with neutrophiles and eosinophiles and mild reticulo-endothelial proliferation; Section 3: accumulation of eosinophiles in medulla: Section 4: erythrocytic phagocytosis.

Spleen - capsular siderosis; mild lymphocytic hypoplasia.

Prostate - scattered foci of subacute prostatitis; several areas of vicarious lymph node formation with definitive germinal centers (mitotic figures numerous).

Lung - single focal area of eosinophilic infiltration with proliferation of septal cells; several foci of mild hemorrhage; fibrinous exudate in alveoli.

APPENDIX 2

CARBON TETRACHLORIDE DOGS

DOG 6664

Beagle Cross Female BSP retention: 21.8%

Gross Pathology:

Emaciated; two circumscribed, necrotic foci, 3.0 cm. in diameter, one on the buccal surface of each cheek, covered by a fibrinonecrotic membrane; focal hepatization and generalized edema of the lungs; liver yellowish brown with irregular mottling (nutmeg appearance); spleen small and pale.

Histopathology:

Liver - severe centrolobular degeneration characterized by disruption of cordal architecture with vacuolization of the hepatocellular cytoplasm, bizarre nuclei, occasional necrotic foci and congestion involving 2/3 of the lobule; minimal inflammatory response; peripherolobular cloudy swelling and frequent vacuolization; diffuse congestion; severe centrolobular fatty metamorphosis.

Lung - acute focal bronchopneumonia and edema.

Kidneys - cloudy swelling of the convoluted tubules; vacuolization of the collecting tubules; numerous casts.

Lymph nodes - medullary edema and lymphoid hyperplasia.

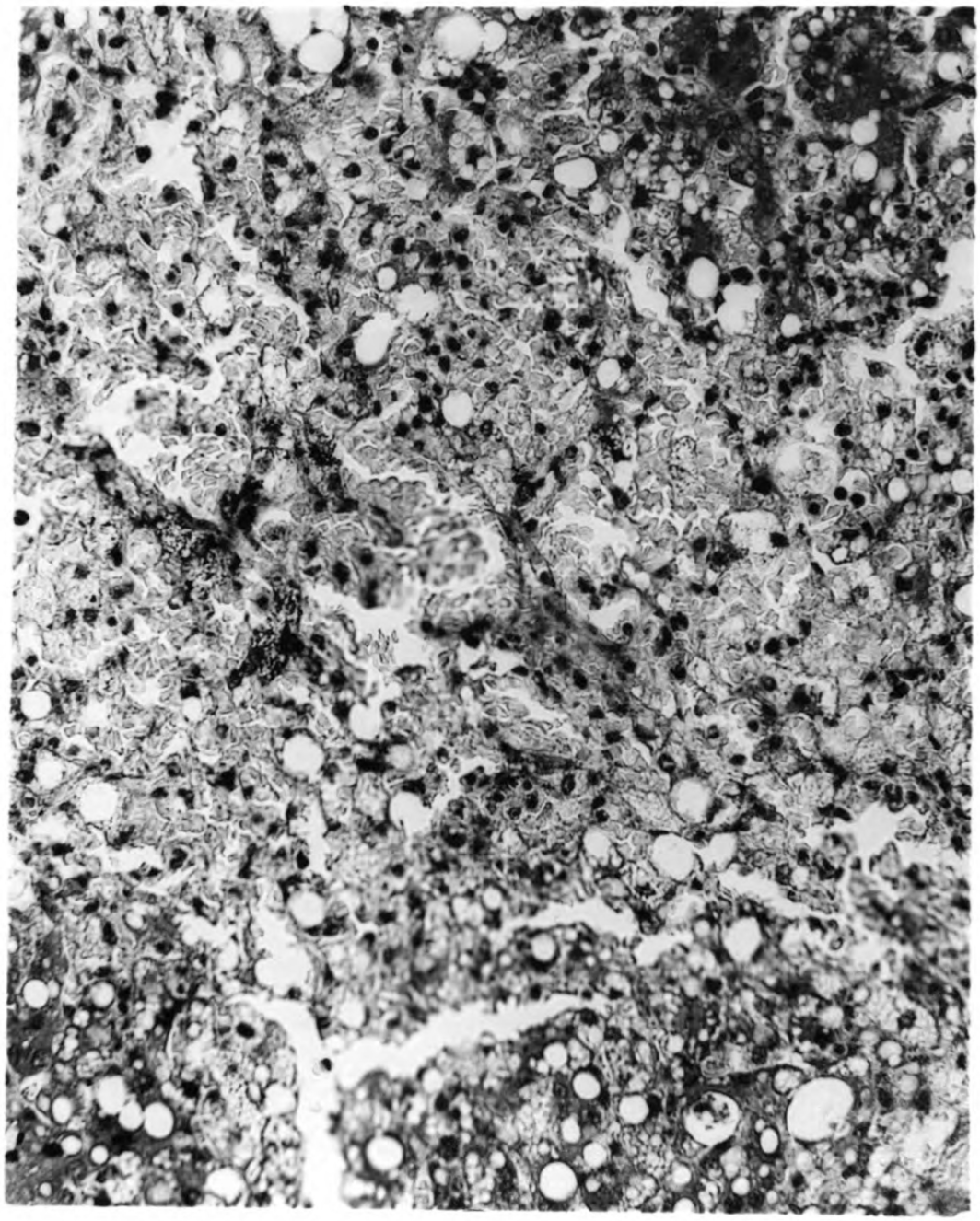
DOG 6796

Beagle Cross Male BSP retention: 0.8%

Gross Pathology:

Marked obesity; multiple, elevated red or gray nodules, 2-3 cm. in diameter, on the posterior border of the spleen; thyroids enlarged

Fig. 1. Liver. Dog 6664. Carbon tetrachloride toxicosis. Severe centrolobular congestion, disruption of cordal architecture, and pycnotic nuclei; periphrolobular fatty metamorphosis, cloudy swelling, and focus of regenerating hepatic cells. X400.



and gray on cut surface.

Histopathology:

Liver - generalized hepatocellular swelling with distortion of the cells in several areas; frequent mild to moderate proliferation of fibroblasts and infrequent lymphocytic cuffing at the central veins; generalized bile stasis in the small bile canaliculi around the central veins; numerous individual cells with sudanophilic inclusions, centrolobularly.

Spleen - lymphoid hyperplasia with many of the germinal centers replaced by epithelioid cells or small hemorrhages; focal capsular hemorrhage; abundant pigment, free and phagocytized.

Thyroid - unilateral, diffuse subacute thyroiditis.

DOG 7071

Tan Mongrel Male BSP retention: 34.0%

Gross Pathology:

Numerous, small, white scars or bullae and several small hemorrhages on both lungs; spleen rough with multiple marginal white nodules; moderate ascites; numerous white subcapsular scars, 2 mm. in diameter, in both kidneys; gastric mucosa congested; hepatic lymph nodes juicy and dark red in color; mucosa of duodenum and anterior segment of jejunum moderately congested; liver slightly atrophic, yellowish with deeper yellowish mottling, with a finely pebbled appearance, cuts with resistance; cholecyst frosty in appearance on mucosal surface; fine petechiae in neck of urocyst.

Histopathology:

Liver - distinct lobulation delineated by early perilobular fibrosis;

hepatocellular necrosis with hemorrhage - the latter predominating in extent over the fibrotic reaction; slight infiltration with lymphocytes, macrophages and occasionally neutrophiles; mild proliferation of the endothelium; occasional new bile duct formation; all hepatic cells unusually acidophilic; cordal architecture deranged with dilatation of sinusoids and bile canaliculi; several scattered areas of phagocytized pigment (probably hematogenous); scattered foci of regenerating hepatic cells.

Hepatic lymph node - medullary sinuses dilated and filled with erythrocytes and erythrophagocytotic macrophages; cortex slightly atrophic with areas of phagocytized pigment similarly found in several large trabeculae.

Mesenteric lymph node - moderate lymphoid hyperplasia.

Stomach - mild congestion of mucosa.

Spleen - capsular siderosis; abundant free and phagocytized hematogenoid pigment; increase in stromal cells of red pulp.

Urocyst - moderate hyperemia in proximal part of submucous layer.

Kidney - abundant intracellular pigment in tubule cells; abundant acidophilic and basophilic amorphous material in lumens; many glomeruli contained acidophilic granular material.

Cholecyst - adherent polychromic, fibrinoid material on the mucosa.

DOG 7072

Black and Gray Mongrel Female BSP retention: 29.8%

Gross Pathology:

Two large hemorrhages on left suprapharyngeal lymph node; marked congestion of urethra; vagina congested; duodenal mucosa congested;

liver mildly congested and with very distinct interlobular septa; cholecyst wall markedly edematous; gastric mucosa congested.

Histopathology:

Liver - microscopic picture primarily that of degeneration; glycogen laden cells especially prominent periportally; focal hemorrhage with residuum of hematogenoid pigment; slight increase in periportal fibrosis; centrolobular hemorrhage, mild fibrosis and pigment accumulation (chiefly in Kupffer cells); scattered areas of interlobular fibrosis in perilobular connective tissue; inflammatory reaction scant and consisting primarily of lymphocytic infiltration; cloudy swelling and acidophilia of remaining hepatic cells; disruption of cordal architecture from dilated sinusoids and bile canaliculi.

Spleen - capsular siderosis at hilus; abundant pigment, free and phagocytized.

Stomach - mild congestion of mucosa; mild edema just below the mucous glands.

Cholecyst - marked intra-adventitial edema.

Kidneys - abundant amorphous material in lumens of tubules; mild hypertrophy of Bowman's capsule.

Suprapharyngeal lymph node - single focal hemorrhage in cortex.

Hepatic lymph node - moderate medullary hemorrhage.

Spleen - splenic corpuscles depleted; lymphoid exhaustion in parenchyma.

Urocyst - marked, distinct, hyperplasia of fibroblasts in submucous layer; luminal surface of transitional epithelium flattened and with a thin hyperkeratotic band.

DOG 7073

Tan Mongrel Female BSP retention: 23.6%

Gross Pathology:

Moderate ascites; liver small, uneven, with numerous, almost clear, elevated nodules on the surface - yellow and red mottling - firm and cuts with resistance; hepatic lymph node congested; edema of the mesentery near the hilus of the liver and involving the pancreas.

Histopathology:

Liver - distinct lobulation from perilobular fibrosis, and vascular dilatation and hemorrhage in interlobular connective tissue; diffuse glycogen infiltration involving practically the entire lobule leaving only an incomplete border of regenerating or albuminous degenerating hepatic cells; frequent bile duct proliferation not necessarily confined to the islands of Glisson; inflammatory reaction scant and primarily lymphocytic; abundant phagocytized hematogenoid pigment in areas of congestion and hemorrhage; moderage widespread fibrosis.

Pancreas - moderate peripancreatic edema involving also the interlobular connective tissue.

Cholecyst - moderate hyperplasia of the mucosal epithelium.

Kidneys - marked glomerulo-tubular nephrosis.

APPENDIX 3

DRUG TOXICOPATHOLOGY DOGS - BEAGLES

STREPTOVARICIN

30 mg./kg.

DOG 5924

Male BSP retention: 2.2%

Gross Pathology:

Moderate whipworm infection.

Histopathology:

No significant lesions.

DOG 5925

Male BSP retention: 2.2%

Gross Pathology:

Mild, focal congestion of the urocyst; localized area of petechiation in mucosa of the gastric cardio-fundic junction.

Histopathology:

Liver - essentially normal.

Prostate - subacute focal prostatitis.

Pituitary - solitary cyst in pars anterior.

DOG 5926

Male BSP retention: 4.8%

Gross Pathology:

Hepatization of left diaphragmatic lobe of lung; moderate whipworm infestation.

Histopathology:

Liver - essentially normal.

Lung - subacute bronchopneumonia.

100 mg./kg.

DOG 5927

Male BSP retention: 3.6%

Gross Pathology:

Mild, widely distributed, petechiae in ileal mucosa.

Histopathology:

No microscopic lesions (section from ileum normal).

DOG 5928

Male BSP retention: 1.5%

Gross Pathology:

Scar on left apical lobe of lung.

Histopathology:

Liver - excessive number of micro-foci of lymphocytes, not vascularly associated.

Lymph node - marked histiocytic erythrophagocytosis.

Adrenal - multiple, discrete, old hemorrhages in zona reticularis.

Lung - multiple foci of cicatrization and two areas of hyperplasia of metaplastic alveolar epithelium; adjacent pleura thickened by fibrosis.

Kidney - several inear foci of interstitial infiltration with lymphocytes and plasma cells in the vicinity of the thin loops.

DOG 5929

Male BSP retention: 9.0%

Gross Pathology:

Phlegmon, approximately 10 cm. in length, ventral aspect of neck;

cervical lymph nodes enlarged and edematous; suprapharyngeal lymph nodes greatly enlarged and edematous; multiple ecchymotic hemorrhages in cholecyst; moderage hyperemia of gastric fundus; several patches of petechiae in duodenum near pylorus; mild whipworm infection.

Histopathology:

Liver - moderate pavementing in and perivascular infiltration of neutrophiles at the central veins.

Cholecyst - massive hemorrhage in the muscle layer in two areas and perimuscular coat in one area.

Lymph node - diffuse medullary edema.

Pituitary - two large cysts in pars anterior.

Skin - acute suppurative dermatitis and phlegmon of the subcutis.

Thyroid - large areas of follicles with hypertrophic epithelial cells but with an occasional normal follicle within the areas.

TOLBUTAMIDE

30 mg./kg.

DOG 6075

Male BSP retention: 0.8%

Gross Pathology:

Entire length of external surface of small intestine reddish tan in color; liver pale tan in color.

Histopathology:

Liver - essentially normal.

Lymph node - focal deposition of hematogenoid pigment and numerous eosinophiles in the sinuses.

Pituitary - pars anterior polycystic.

Prostate - focal chronic prostatitis.

Stomach - amorphous basophilic material probably calcium surrounded by a thin wall of fibroblasts at irregular intervals mid-zonally in mucosa.

DOG 6076

Female BSP retention: 0.8%

Gross Pathology:

Mild pneumonia of antero-ventral border, right diaphragmatic lobe of lung; liver yellowish in color with focal areas of fine congestion on the surface.

Histopathology:

Liver - no abnormalities in sections examined.

Spleen - capsular siderosis.

DOG 6077

Female BSP retention: 1.5%

Gross Pathology:

One focal area of red hepatization, 3 cm. in diameter, and a diffuse area of induration and compensatory emphysema in right diaphragmatic lobe of lung.

Histopathology:

Liver - essentially normal.

Lung - subacute bronchopneumonia.

Lymph node - hemorrhagic.

100 mg./kg.

DOG 6078

Male BSP retention: 2.2%

Gross Pathology:

Two and a half by one cm. scar on perietal surface, ventral aspect of spleen.

Histopathology:

Liver - essentially normal.

Prostate - chronic focal prostatitis.

Spleen - focal area of trabecular hyperplasia containing small islands of congestion, phagocytosis, abundant pigment and remnants of lymphatic follicles.

Thyroid - marked hypertrophy and hyperplasia of follicular epithelium and infiltration of lymphocytes.

DOG 6079

Female BSP retention: 4.3%

Gross Pathology:

Edema of right vocal cord; mild tapeworm infection.

Histopathology:

Liver - diffuse hepatocellular vacuolization (not fatty metamorphosis).

Kidney - single larval granuloma.

Thyroid - multiple small foci of interstitial infiltration of lymphocytes and plasma cells; epithelium of several follicles slightly hypertrophic.

DOG 6080

Female BSP retention: 1.8%

Gross Pathology:

Cataract, left eye; liver slightly enlarged but otherwise normal.

Histopathology:

Liver - essentially normal.

Thyroid - follicles small with hypertrophic epithelia, reduction in colloid, and marked lymphocytic infiltration and proliferation to point of formation of germinal centers.

160 mg./kg.

DOG 6081

Female BSP retention: 4.3%

Gross Pathology:

Spleen enlarged, congested and the capsule pebbly; hepatic lymph nodes enlarged and edematous; liver enlarged with surface uneven, yellowish mahogany in color and containing yellowish white foci throughout; cholecyst filled with viscid granular bile.

Histopathology:

Liver - widespread micro-foci of distorted hepatic cells, lymphocytes and fibroblasts or of a few necrotic hepatic cells and infiltrating neutrophiles; clusters of lymphocytes throughout the section with dense accumulations around the large bile ducts; hepatocellular vacuolization; peripherolobular fatty metamorphosis.

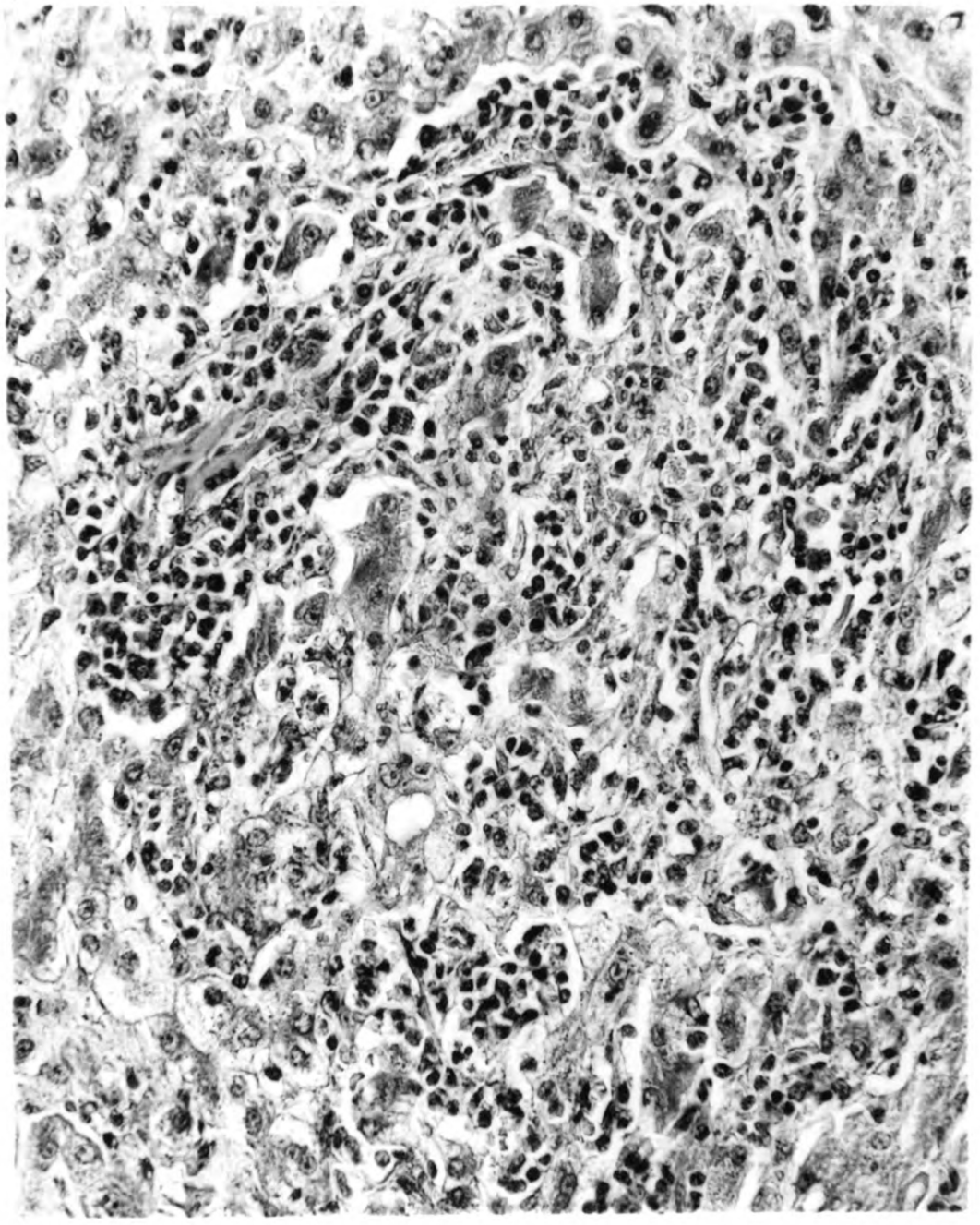
DOG 6082

Female BSP retention: 1.5%

Gross Pathology:

Moderately widespread, white, raised, subcapsular foci, 2 mm. in

Fig. 2. Liver. Dog 6081. Tolbutamide toxicosis.
Focal infiltration with lymphocytes and neutrophiles and atrophy of hepatic cells. X400.



diameter, in cortex of kidney; restricted area of hyperemia and petechiation, proximal 8 cm. of ileum.

Histopathology:

Liver - hepatocellular swelling, otherwise lesions essentially similar to those in Dog 6081 but more neutrophiles present.

Lymph node - medullary congestion.

Kidney - single larval nodule.

Thymus - cystic involution.

Thyroid - several small foci of lymphocytic infiltration; follicles smaller than normal with columnar epithelium.

DIPROPYL-ALLYLOXY-BENZOIC ACID

30 mg./kg.

DOG 6191

Female BSP retention: 2.2%

Gross Pathology:

No gross lesions.

Histopathology:

Liver - a small focus of lymphocytes, angioblasts and regenerating hepatic cells; scattered pigment.

Ileum - neutrophilic infiltration of Peyer's patches and focal acute enteritis.

Lung - early pneumonia.

Pancreas - focal acinar degeneration and occasional necrosis adjacent to or confluent with the islets.

Spleen - capsular siderosis; mild to moderate hematogenoid pigment in the red pulp.

DOG 6192

Male BSP retention: 0.8%

Gross Pathology:

Translucent, hard, verrucous mass on left atrio-ventricular valve.

Histopathology:

Liver - essentially normal.

Spleen - mild hematogenoid pigmentation.

Mandibular lymph node - medullary sinuses markedly infiltrated with eosinophiles; several areas showing cords of proliferating reticulo-endothelial cells.

DOG 6193

Male BSP retention: 0.8%

Gross Pathology:

No gross lesions.

Histopathology:

Liver - essentially normal.

Tonsil - small hemorrhages in many of the germinal centers of the lymphoid follicles.

Spleen - mild hematogenoid pigment accumulation.

Adrenal - small focal hemorrhage in the zona fasciculata.

100 mg./kg.

DOG 6194

Female BSP retention: 0.8%

Gross Pathology:

Moderate roundworm and severe whipworm infestation.

Histopathology:

Liver - mild perivascular eosinophilic infiltration and relative increase in eosinophiles scattered throughout the parenchyma; rectangular area, 500 x 250 microns, consisting of eosinophiles, lymphocytes and fibroblasts.

Kidney - two larval nodules in cortex.

Lymph nodes - moderately infiltrated with eosinophiles; one node, dense accumulation of eosinophiles in medullary sinuses (one of three sections).

Small intestine - notable increase of eosinophiles in the lamina propria of all three sections.

Spleen - abundant eosinophiles and relative increase in neutrophiles.

DOG 6195

Female BSP retention: 0.8%

Gross Pathology:

Two small, white, elevated foci in cortex of kidney; central congestion of the mesenteric lymph nodes.

Histopathology:

Liver - essentially normal.

Mesenteric lymph nodes - sinuses packed with pigment-laden macrophages and eosinophiles; hyperplasia of the lymphoid follicles with dense accumulations of lymphocytes in the sinuses; a few megakaryocytes observed; abundant hematogenoid pigment.

Lymph node - medullary congestion and hemorrhage; moderate hematogenoid pigment.

Spleen - large, canalized thrombus, rich in pigment, and calcifica-

tion of capsule, at the hilus.

DOG 6196

Male BSP retention: 0.8%

Gross Pathology:

No gross lesions.

Histopathology:

Liver - essentially normal.

Prostate - purulent exudate in lumen of two alveoli.

300 mg./kg.

DOG 6197

Male BSP retention: 0.8%

Gross Pathology:

Mild roundworm infestation.

Histopathology:

Liver - essentially normal.

Prostate - diffuse subacute nonsuppurative prostatitis.

DOG 6198

Female BSP retention: 41.2%

Gross Pathology:

Emaciated; generalized peripheral congestion; blood very viscid; adrenals enlarged; lungs edematous with frothy, free-flowing exudate in the trachea - partiel hepatization: apical lobes and left diaphragmatic lobe, complete in: cardiac lobes, azygos lobe and right diaphragmatic lobe; stomach markedly dilated (atony), congested and containing a large quantity of gelatinous exudate; pancreas intensely congested; edema of the external surface of the intestines; entire tract was empty; spleen shrunken; congestion of

mesenteric lymph nodes; perirenal edema of right kidney and kidneys slightly congested with yellowish cast and dark cortices; liver purplish cast with yellow mottling and very fine, dark, subcapsular, foci without a further pattern of distribution; cholecyst engorged; all viscera mildly congested and faintly yellow in color.

Histopathology:

Liver - disruption of hepatic cords from congestion of the sinusoids, cloudy swelling peripherally and fatty metamorphosis centrolobularly.

Adrenals - mild congestion.

Cholecyst - severe autolysis of mucosa.

Brain - dilatation and perivascular edema of the fissural veins.

Kidney - mild passive congestion with tubular cytoplasm granular and vacuolated; areas of cloudy swelling.

Lung - diffuse alveolar congestion; perivenous edema; mild infiltration of neutrophiles whose distribution is diffuse and not that of typical bronchopneumonia; areas of edema and compensatory emphysema; basophilic granular material suggesting aspirated foreign material in a number of alveoli in one section.

Suprapharyngeal lymph node - marked depletion of follicles; subcapsular sinuses stuffed with neutrophiles that also infiltrated the medullary sinuses; edema.

Mesenteric lymph node - venous congestion; germinal centers of follicles practically abolished; proliferation of macrophages; sinuses packed with lymphocytes.

Spleen - trabeculae prominent; paucity of red pulp; moderate hypoplasia of follicles; focal hyperemia; moderately abundant hematogenoid pigment.

Stomach - muscular coat thin, mucosa diminished in depth and the mucous glands dilated with secretion; parietal cells swollen and cytoplasm of chief cells disrupted.

Pancreas - moderate venous congestion.

Tonsil - germinal centers replaced, centrally, by lymphoblasts or macrophages and collagen or hyaline-like material - young and mature lymphocytes present in gland.

DOG 6199

Male BSP retention: 0.8%

Gross Pathology:

No gross lesions.

Histopathology:

Liver - essentially normal.

Cerebellum - focal area of encephalomalacia involving molecular layers in two apposing gyri with proliferation of microglia and astrocytes; some glial cells undergoing karyolysis as well as those in the glanular layer.

Kidney - moderate, focal, subacute pyelitis.

Prostate - mild, focal, subacute prostatitis.

Spleen - capsular siderosis; mild pigment accumulation.

Urocyst - mild, focal, subacute cystitis.

PYRAZOLIDIN-3-ONE, 1-METHYL-5-PHENYL

30 mg./kg.

DOG 6246

Female BSP retention: 9.8%

Gross Pathology:

Liver mottled throughout (nutmeg appearance) with greenish cast

and increased firmness; small cyst in anterior lobe of pituitary; severe tapeworm infestation.

Histopathology:

Liver - diffuse, moderately intense and predominately centrolobular, deposition of hematogenoid pigment in small to large globular masses; Kupffer cells in area pigment-laden; slight increase in perivascular cells (lymphocytes and neutrophiles); commonly, hypertrophy of endothelial cells of central veins or, less commonly, rhexis of the vein with small, confined hemorrhage; mild, centrolobular, fatty metamorphosis; lesions involved approximately 25-33% of section.

Bone marrow - abundant pigment not hemosiderin; mild decrease in myelocytes.

Lung - solitary calcifying granuloma.

Pituitary - small mucoid cyst in pars anterior.

Spleen - moderate hematogenoid pigment accumulation; reticulum cell hyperplasia at periphery of corpuscles.

DOG 6247

Female BSP retention: 6.2%

Gross Pathology:

Liver mahogany colored; areas of congestion scattered throughout the small intestine; small focal hemorrhages in mucosa of urocyst.

Histopathology:

Liver - extensive but mild, predominantly centrolobular, accumulation of pigment as fine to large globules; Kupffer cells not pigment bearing to any significant degree; hypertrophy of small bile duct epithelium almost obliterating the lumens at the triads;

perivascular accumulations of lymphocytes and neutrophiles slight to normal; mild, centrolobular, fatty metamorphosis; approximately 10% of section area involved.

Bone marrow - abundant pigment (not hemosiderin); relative decrease in myelocytes.

Lymph node - small follicular hemorrhage; two confluent foci of eosinophiles with numerous mitotic figures in lymphocytes in the medulla.

Pituitary - small mucoid cysts in pars anterior.

100/60 mg./kg.

DOG 6243

Male BSP retention: 21.8%

Gross Pathology:

Emaciated; generalized muscular atrophy; edema of the subcutis; liver nutmeg in appearance, slightly bronzed and cuts with increased resistance; spleen enlarged with mottled surface and irregular areas of congestion; lymph nodes edematous; kidneys pale, friable, and with a few white subcapsular foci (3-4 mm); myocardial hypertrophy and dilatation of the heart; testes atrophic; catarrhal enteritis and bile-staining of the mucosa of the small intestine; cyst in anterior lobe of pituitary; marginal consolidation (4-5 mm) of the lungs.

Histopathology:

Liver - generalized dilatation of the sinusoids, centrolobularly especially, with atrophy of adjacent hepatic cords and fatty degeneration and necrosis at the central vein; scattered foci of lymphocytes and neutrophiles in the dilated sinusoids; scattering of fine

pigment, intra-and extracellularly, throughout and larger particles located centrolobularly - in these areas, infiltration of lymphocytes and fibroblasts, and disorganization of reticular fibers of stroma; marked fatty metamorphosis centrally with involvement of 1/2 to 2/3 of the lobule; approximately 50% of the section area involved.

Spleen - corpuscles indistinct and showing marked atrophy, paucity of lymphoid cells with frequent pycnotic nuclei, hyalinization of intercellular substance, moderate hematogenoid pigment accumulation; and areas resembling pale infarcts.

Lymph nodes - loss of follicular pattern in some nodes; accumulation of hemosiderin in cortical follicles.

Testis - spermatogenesis retarded chiefly at secondary spermatocyte stage; numerous mutinucleated giant cells in lumens of tubules.

Pancreas - atrophy of peripheral acini.

DOG 6244

Female BSP retention: 10.5%

Gross Pathology:

Liver faintly greenish in color but mottled throughout and firm; adrenal slightly enlarged; spleen enlarged; consolidation of right cardiac lobe of lung; mild roundworm infection.

Histopathology:

Liver - predominantly centrolobular deposition, intra- or extracellular, of pigment and commonly in large droplets; in associated areas, hepatocellular atrophy, vacuolization and hydropic degeneration and dilatation of the sinusoids; inflammatory cell infiltration infrequent and consisting essentially of eosinophiles, with

lymphocytes and a few fibroblasts; edema around the portal and hepatic veins; 2/3 of lobule involved; approximately 50% of section area involved.

Spleen - mild lymphoid hypoplasia and moderate pigment accumulation.

Adrenal - moderate cortical hyperplasia.

Lung - perivascular edema and hemorrhage around larger pulmonary vessels.

Lymph node - pigment in follicles; erythrophagocytosis.

Bone marrow - abundant pigment (not hemosiderin) and moderate myelocytic hypoplasia.

Pituitary - mucoid cyst, 1 mm in diameter, in pars anterior.

DOG 6245

Female BSP retention: 16.2%

Gross Pathology:

Spleen enlarged and congested; few white subcapsular foci in both kidneys; liver congested, slightly mottled, firm and with wrinkling or pitting of the surface; pancreatic and gastric lymph nodes congested; small cyst in dorsum of anterior lobe of pituitary.

Histopathology:

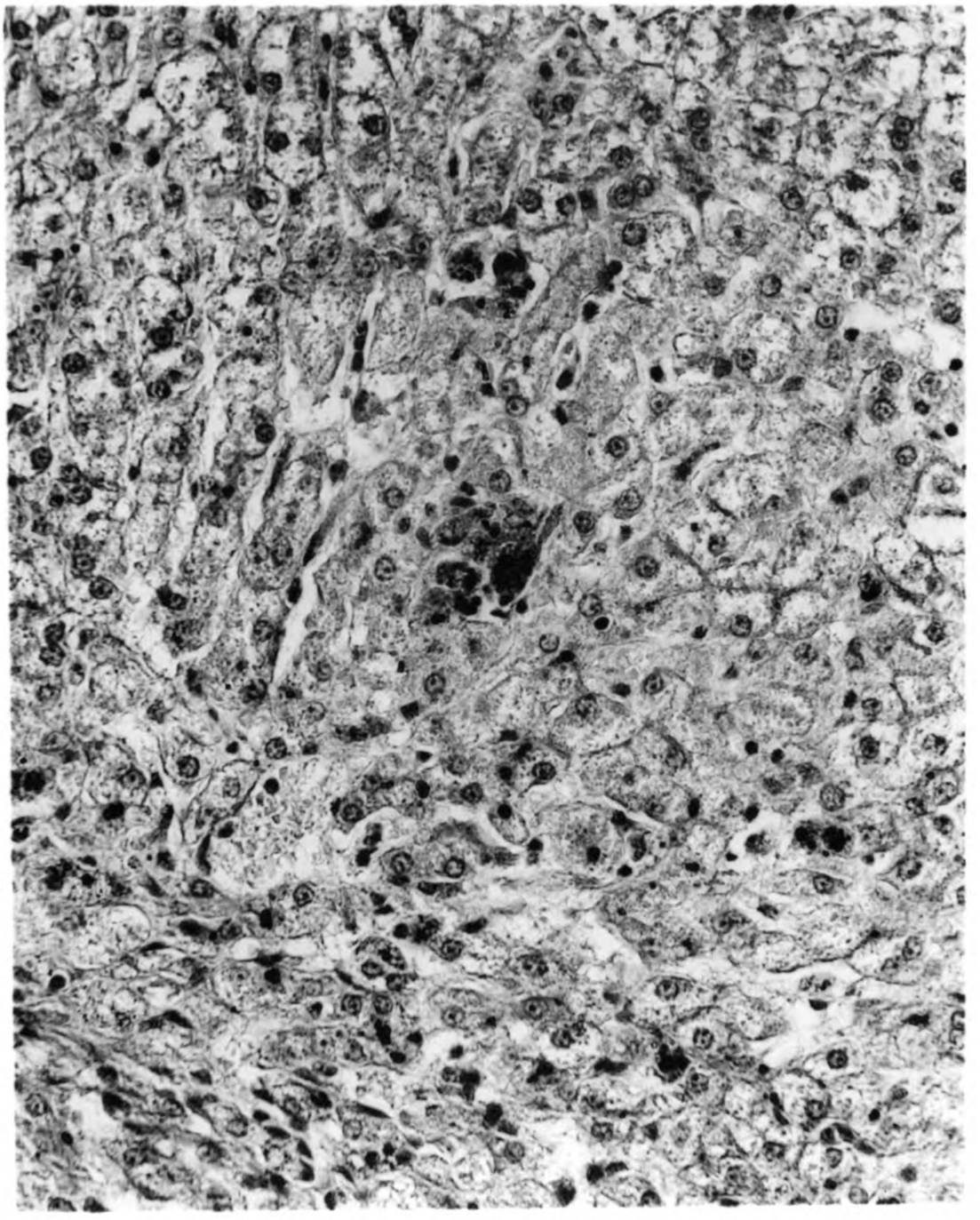
Liver - lesions essentially similar to those in Dog 6244 but hepatocellular degeneration more intense, bordering on frank necrosis, at the central vein; mild centrolobular fatty metamorphosis; approximately 50% of section area involved.

Spleen - congested and moderate pigment accumulation.

Bone marrow - abundant pigment (not hemosiderin) and moderate myelocytic hypoplasia.

Fig. 3. Liver. Dog 6245. Pyrazolidin-3-one, 1-methyl-5-phenyl toxicosis.

Centrolobular cloudy swelling and deposition of pigment in fine and large droplets, both intra- and extracellularly. X400.



DOG 6241

Female BSP retention: 7.0% (1:45/hrs.)

Gross Pathology:

Small organized hemorrhage on tricuspid valve of heart; liver firm with dark pebbling; kidneys congested; adrenals enlarged; mucosa of stomach stained with bile; spleen congested and firm.

Histopathology:

Liver - passive congestion, moderate centrolobular accumulation of pigment, both fine and large particles, with marked atrophy of cord cells many of which showing degeneration of cytoplasm and necrosis; mild edema; generalized foci of fine sudanophilic material but larger and more numerous at the central vein; approximately 75% of section involved.

Spleen - moderate accumulation of pigment and reticulum cell hyperplasia.

Kidneys - marked congestion of glomeruli and calcific plugs in some collecting tubules in medulla.

Heart - deposition of finely dispersed fat in myocardial fibers.

Pituitary - small mucoid cyst in pars anterior.

METHYL RESERPATE

1 mg./kg.

DOG 6470

Female BSP retention: 0.8%

Gross Pathology:

Lateral ventricles enlarged indicating moderate hydrocephalus.

Histopathology:

Liver - section 1: one small focus of lymphocytes, eosinophiles and fibroblasts; section 2: several triads exhibit infiltration with lymphocytes, eosinophiles, macrophages and a few plasma cells - lesions small, confined, and very mild.

Kidneys - section 1: one focus of lymphocytes, with some fibroblasts, around regenerating tubules in the cortex; section 2: two foci of lymphocytes at the junction of the medulla and pelvis.

Lymph node - medulla filled with eosinophiles; localized accumulation of pigment in large masses, with many pigment-laden macrophages.

DOG 6471

Female BSP retention: 0.8%

Gross Pathology:

In estrus; mucosa of neck of urocyst congested; low breaking strength of ribs.

Histopathology:

Liver - essentially normal.

Lymph node - medullary sinuses profusely infiltrated with eosinophiles and proliferating reticulo-endothelial cells; masses of pigment at one pole.

Spleen - moderate accumulation of pigment.

Uterus - degenerating endometrium.

5 mg./kg.

DOG 6463

Female BSP retention: 0.8%

Gross Pathology:

Obese; epidermis of rear paws thin; complete consolidation of

azygos lobe and partial consolidation of cardiac and diaphragmatic lobes of right lung; bronchial lymph nodes swollen and congested; spleen swollen and plum-colored; intracapsular cysts in both kidneys, rickets.

Histopathology:

Liver - essentially normal.

Kidneys - mild subacute interstitial pyelitis in one and moderate subacute pyelonephritis in the other kidney; in one section, area approximately .1 mm. in diameter comprises 3 defective glomeruli and a small focus of lymphocytes and proliferating fibroblasts along the tubular tracts.

Heart - very fine sudanophilic particles in tail-like conformation directly behind or in front of the nucleus of the myocardial cells involving a rather extensive area beginning at the endocardium and extending deep into the myocardium.

Tonsil - mild purulent tonsillitis.

Lung - section 1: scattered areas of inflammatory cells concentrated primarily peribronchiolarly although several foci are located in alveoli - eosinophiles primary cell with macrophages secondary and lymphocytes scanty - many single eosinophiles randomly distributed throughout the lung parenchyma; section 2: one focus of lymphocytes, macrophages, eosinophiles and neutrophiles around a foreign body.

Lymph node - subcapsular, circumscribed, eosinophilic granuloma.

Spleen - marked congestion.

Stomach - multiple solitary lymph nodules with active germinal centers in the mucosa.

DOG 6464

Female BSP retention: 0.8%

Gross Pathology:

Thyroids slightly enlarged; spleen swollen and plum-colored; urocyst slightly congested.

Histopathology:

Liver - essentially normal.

Lymph node - section 1: mildly hyperactive germinal centers; section 2: mild hyperplasia of follicles with mild infiltration of medullary sinuses peripherally (near the cortex) with eosinophiles.

Tonsil - mildly hyperactive germinal centers.

Thyroid - lymphocytic proliferation, primarily at the periphery of the organ but moderately abundant in the central portion, consisting almost exclusively of young lymphocytes with only a few scattered concentrations of mature cells; follicles generally not affected except in areas of lymphocytic proliferation causing them to undergo pressure atrophy leading to complete obliteration.

Spleen - marked congestion and mild hypoplasia of Malpighian corpuscles.

Adrenal - excessive sudanophilic inclusions in zona glomerulosa.

15 mg./kg.

DOG 6465

Male BSP retention: 0.8%

Gross Pathology:

Sacrificed in extremis; emaciated; generalized venous congestion; fundament smeared with tan fecal material; liver slightly shrunken with yellowish cast and congested areas; heart slightly enlarged

flabby; pancreas congested; spleen smooth and congested; mild infestation with whipworms; irregular congestion in extreme anterior segment of duodenum; linear congestion of colon; muscles atrophic; posterior parathyroids enlarged.

Histopathology:

Liver - essentially normal.

Colon - mild venous dilatation in mucosal vessels at apex of several plicae.

Lymph node - sinuses stuffed with reticulo-endothelial cells.

Pancreas - pancreatic islets appear smaller than normal.

Prostate - chronic interstitial proliferation.

Spleen - intense congestion with moderate follicular hypoplasia.

Tonsil - acute purulent tonsillitis.

DOG 6466

Female BSP retention: 0.8%

Gross Pathology:

Excoriation of rear foot pads; epidermis of forepaws thin but intact; hind parts matted with dried, black, fecal material; diarrhea; vulva relaxed; spleen smooth, large, swollen and plum-colored; pieces of paper carton in stomach; slight congestion of mucosa of urocyst; rickets.

Histopathology:

Liver - essentially normal.

Kidneys - heavy, almost exclusively intracellular, deposition of orange pigment in convoluted tubules of both kidneys - collecting tubules and glomeruli spared; several glomeruli swollen with clumps of sudanophilic material within.

Lymph node - mild follicular hypoplasia; extensive erythrophagocytosis.

Spleen - marked congestion; moderate hypoplasia of Malpighian corpuscles.

APPENDIX 4

DOGS WITH CANINE DISTEMPER

DOG 6334-1

Black Cocker Cross Female BSP retention: 0.8%

Clinical:

Temperature, 103.4°F; serous nasal discharge; slight ocular discharge; sclera injected; induced cough; slightly increased vesicular murmur.

Gross Pathology:

Pulmonary lymph nodes congested; spleen pale and thick but of normal length and width.

Histopathology:

Liver - sinusoids slightly dilated; hepatic cords slightly atrophic.

DOG 6334-2

Balck and White Collie Cross Male BSP retention: 2.5%

Clinical:

Temperature, 103.8°F; profuse mucopurulent nasal discharge; harsh cough.

Gross Pathology:

Spotty red hepatization, right cardiac lobe; multiple, fine, white foci in the kidneys; yellowish cast to the liver; no food in stomach; intestines empty.

Histopathology:

Liver - frequent individual cells undergoing karyolysis adjacent to the central vein and mid-lobularly; scattered Kupffer cells pigment-laden; intranuclear salt inclusions conspicuous near central veins.

DOG 6334-3

Brindle Mongrel Male BSP retention: 4.8%

Clinical:

Temperature, 104.4°F; profuse mucopurulent nasal discharge; sclera injected; induced cough.

Gross Pathology:

Diffuse pulmonary congestion and hepatization, right cardiac lobe; liver congested, but with large yellowish areas; mucosa of urocyst congested; stomach and intestines empty.

Histopathology:

Liver - dilatation and congestion of sinusoids centrolobularly with atrophy of adjacent hepatic cords and small nidi of proliferating Kupffer cells; hepatic cells undergoing cloudy swelling and fatty metamorphosis in more severely affected areas; throughout sections, infrequent cells exhibiting karyolysis or pycnosis of nuclei in areas adjacent to central veins.

Spleen - splenic lymphoid follicles indistinct and germinal centers practically abolished; mild, diffuse infiltration with neutrophiles and reticulo-endothelial cells prominent.

Lung - acute nonsuppurative bronchopneumonia.

Kidney - mild dilatation of the collecting tubules.

DOG 6337-543

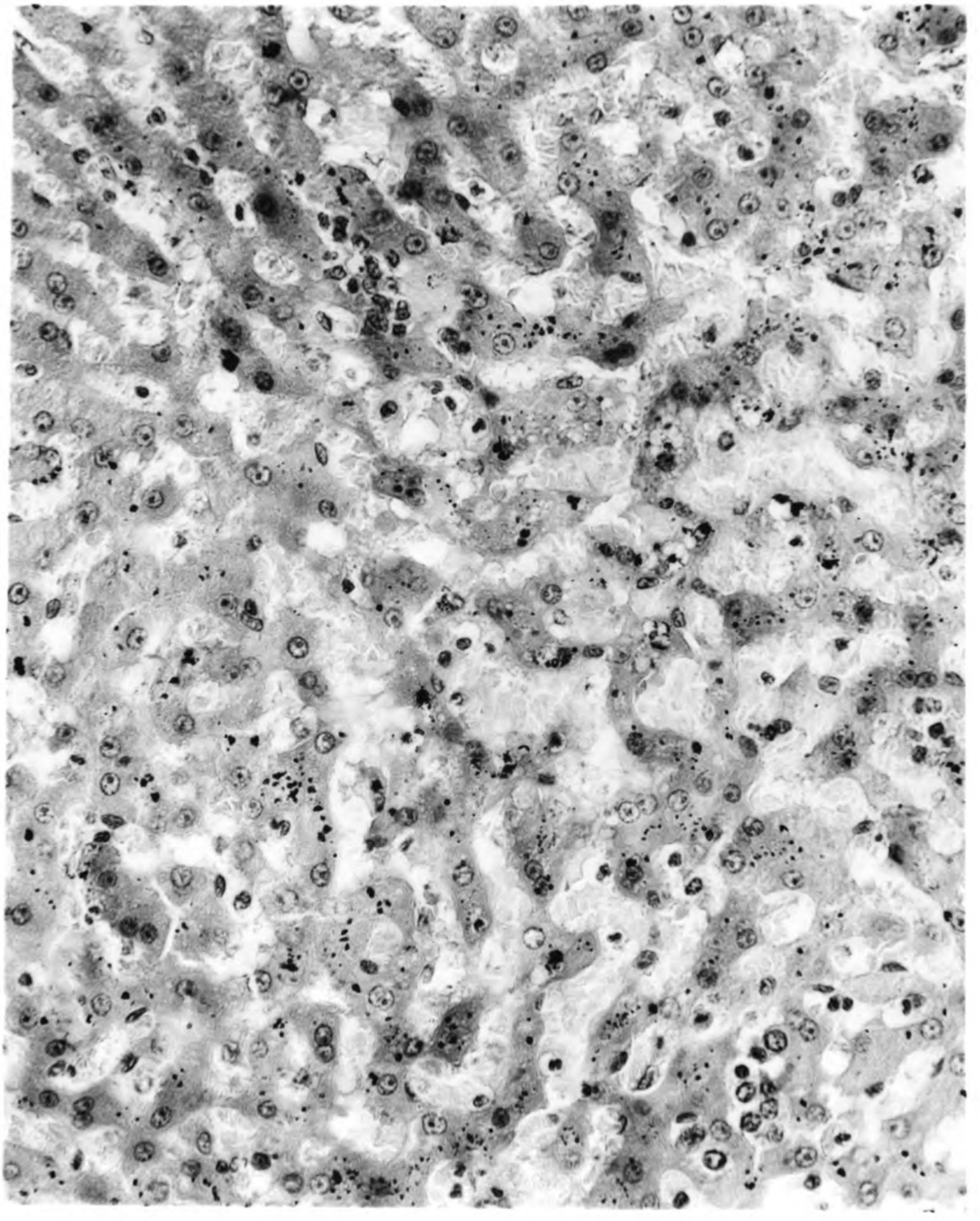
Beagle Male BSP retention: 0.8%

Clinical:

Temperature, 103.6°F; profuse purulent ocular and nasal discharge; increased vesicular murmur; sclera injected.

Gross Pathology:

Fig. 4. Liver. Dog 6334-3. Canine Distemper Complex. Centrolobular dilatation and congestion of the sinusoids and atrophy of adjacent hepatic cords. The fine pigment in the cells is artifact. X400.



Nares occluded with dried exudate; mucopurulent exudate in conjunctival sac; gastrointestinal tract empty.

Histopathology:

Liver - sinusoids slightly dilated in some but not all lobules at central veins and infrequent individual hepatic cells exhibiting karyolysis in some areas; mild cloudy swelling; few scattered Kupffer cells are pigment-laden.

Lung - healing pneumonia with fibrosis prominent.

Stomach - gastric pits distended with mucus.

Prostate - mild interstitial fibrosis.

Turbinates - mild purulent rhinitis.

DOG 6337-4

Black Cocker Cross Male BSP retention: 0.8%

Clinical:

Temperature, 103.4°F; harsh tracheal sounds; sclera injected; purulent ocular discharge; serous nasal discharge; sneezing.

Gross Pathology:

Emaciated; tonsils slightly congested; mandibular and suprapharyngeal lymph nodes enlarged, edematous and irregularly congested; nasal mucosa congested and covered with purulent exudate; liver congested; roundworms, tapeworms and whipworms in intestines; gastrointestinal tract devoid of ingesta.

Histopathology:

Liver - mild passive congestion and cloudy swelling.

Lymph nodes - several cortical hemorrhages; germinal centers indistinct and cortex and medulla solidly cellular with lymphocytes and proliferating reticulo-endothelial cells.

Spleen - acute splenitis.

Nictitating membrane - multinucleated giant cells present.

Trachea - a few intracytoplasmic inclusion bodies.

DOG 6337-5

Black and White Mongrel Male BSP retention: 1.2%

Clinical:

Temperature, 103.1°F; slight purulent ocular discharge; sclera injected; moderate purulent nasal discharge; mild diarrhea.

Gross Pathology:

In poor flesh; hemorrhages in left tonsil; consolidation of part of right cardiac and posterior edge of right apical and entire left apical lobes; liver congested; congestion of prostate; small varices in urocyst; gastro-intestinal tract empty; light infestations with hookworms, tapeworms and whipworms.

Histopathology:

Liver - cloudy swelling of hepatic cells and scattered Kupffer cells pigment-bearing.

Lung - subacute purulent bronchopneumonia.

Urocyst - a solitary, diffuse hemorrhage in submucosal layer.

Kidney - bilateral subacute pyelitis, mild in one kidney and moderate in the other.

Prostate - focal subacute prostatitis.

DOG 6337-6

Beagle Female BSP retention: 0.8%

Clinical:

Temperature, 103.4°F; mucopurulent nasal discharge; cough; sclera

injected; dull areas on auscultation; mucoid diarrhea.

Gross Pathology:

In fair flesh; mucopurulent nasal exudate; mucoid exudate in trachea; consolidation of lower half of right cardiac lobe of lung, postero-ventral portion of left apical lobe and entire left cardiac lobe; one mature Dirofilaria immitis in right ventricle of heart and one small dirofilaria in pulmonary artery; solitary diffuse hemorrhage in urocyst; congestion of gastric mucosa; multiple, subcapsular, white foci, 2 mm in diameter, in both kidneys; mild infestation of roundworms and whipworms; nasal cavity occluded with mucopurulent exudate and congestion of mucosa.

Histopathology:

Liver - scattered pigment-bearing Kupffer cells in all sections; one small oval area of fibrosis in one section.

Lung - diffuse, acute, purulent bronchopneumonia.

Trachea - intracytoplasmic inclusion bodies.

Spleen - acute nonsuppurative splenitis.

Kidneys - unilateral subacute nonsuppurative pyelitis and one small larval granuloma.

Urocyst - mild acute cystitis with mild erosion of the mucosa; intracytoplasmic inclusion bodies distinct.

Lymph node - subacute lymphadenitis.

Membrana nictitans - subacute proliferative conjunctivitis.

Stomach - mucosa flattened and mucous glands abbreviated.

DOG 6337-7

Black and Brown Mongrel Male BSP retention: 0.8%

Clinical:

Temperature, 102.1°F; sclera slightly injected; dried exudate on external nares; cough.

Gross Pathology:

Liver paler than normal; multiple, diffusely distributed small white foci, located subcapsularly and in cortex of kidneys; gastro-intestinal tract empty except for small tapeworms, a few roundworms and many whipworms.

Histopathology:

Liver - 1st section: one fine focus of lymphocytes, fibroblasts and eosinophiles; one focus, irregular in outline but sharply circumscribed, composed of lymphocytes, eosinophiles, fibroblasts and a few neutrophiles surrounding several new capillaries; lumen of adjacent small vein completely obliterated by proliferating endothelium and circumscribed by thick layer of lymphocytes, eosinophiles, degenerating hepatic cells, macrophages and new capillaries; increased perivascular cuffing; approximately 1/300 of total section involved. 2nd section: one area containing multiple adjacent foci, either circumscribed of spreading, consisting of focal necrosis, infiltration of lymphocytes, eosinophiles and macrophages with few neutrophiles and proliferation of fibroblasts; approximately 1/40 of total section involved.

Kidney - moderate bilateral subacute pyelonephritis; larval granuloma; several areas of chronic nephritis.

Membrana nictitans - subacute conjunctivitis.

DOG 6337-8

Black and Tan Mongrel Female BSP retention: 6.2% (mild hemolysis)

Clinical:

Temperature, 103.6°F; mucopurulent ocular and nasal discharge; sclera injected; serosanguinous exudate from mouth (severe bilateral necrotic stomatitis); emaciated.

Gross Pathology:

Emaciated; fetid, sanguinopurulent exudate on the lips and circumoral hair; at angle of the jaws, on the buccal surface of the cheek, an ulcerated area (24 mm in diameter) with raised, ragged edges and with fibrinonecrotic membrane; manidibular lymph nodes muddy gray in color and irregularly congested; heart enlarged, flabby and with thin myocardium; irregular areas of atelectasis, gray in color, in cardiac lobe of lung; slight yellowish discoloration of liver; blood viscid; gastro-intestinal tract devoid of ingesta.

Histopathology:

Liver - loss of cord structure with loss of cell architecture and karyolysis common, especially centrolobularly; frequent vacuolization of hepatic cell cytoplasm; many solitary polymorphs in the sinusoids; clusters of two or three Kupffer cells frequent in the same location; peripholobularly, cloudy swelling and vacuolization; lesions present throughout section.

Spleen - acute splenitis.

Lymph node - lymphocytic depletion; node solidly cellular.

Kidneys - mild bilateral pyelitis.

DOG 6337-9

Black Cocker Cross Female BSP retention: 9.8%

Clinical:

Temperature, 105.0°F; emaciated; sibilant rales on auscultation;

mucopurulent ocular and nasal discharge; sclera injected; pupils dilated; diarrhea, with fluid, green, blood-tinged feces; cough.

Gross Pathology:

Emaciated; external nares occluded with dried exudate; mucopurulent exudate in conjunctival sac; red hepatization of lower 1/3 of right apical and diaphragmatic lobes of lung, entire right cardiac lobe and lower third of all left lobes; pulmonary lymph nodes enlarged and congested; yellowish color to entire liver with areas of more distinct yellowish mottling; kidneys slightly congested; nasal mucosa congested; tonsil congested; gastro-intestinal tract empty.

Histopathology:

Liver - multiple lesions throughout section, consisting of two distinct entities: a) at the central vein, rhexis of the vein with hemorrhage, congestion of adjacent sinusoids, accumulation of hematogenoid pigment and degeneration of adjacent hepatic cells; b) well demarcated, approximately round or oval in shape, foci of necrosis with infiltration of neutrophiles and macrophages and hemorrhage, not necessarily centrolobular; approximately fifty percent of the total area of sections involved with both types of lesions.

Urocyst - intracytoplasmic inclusion bodies.

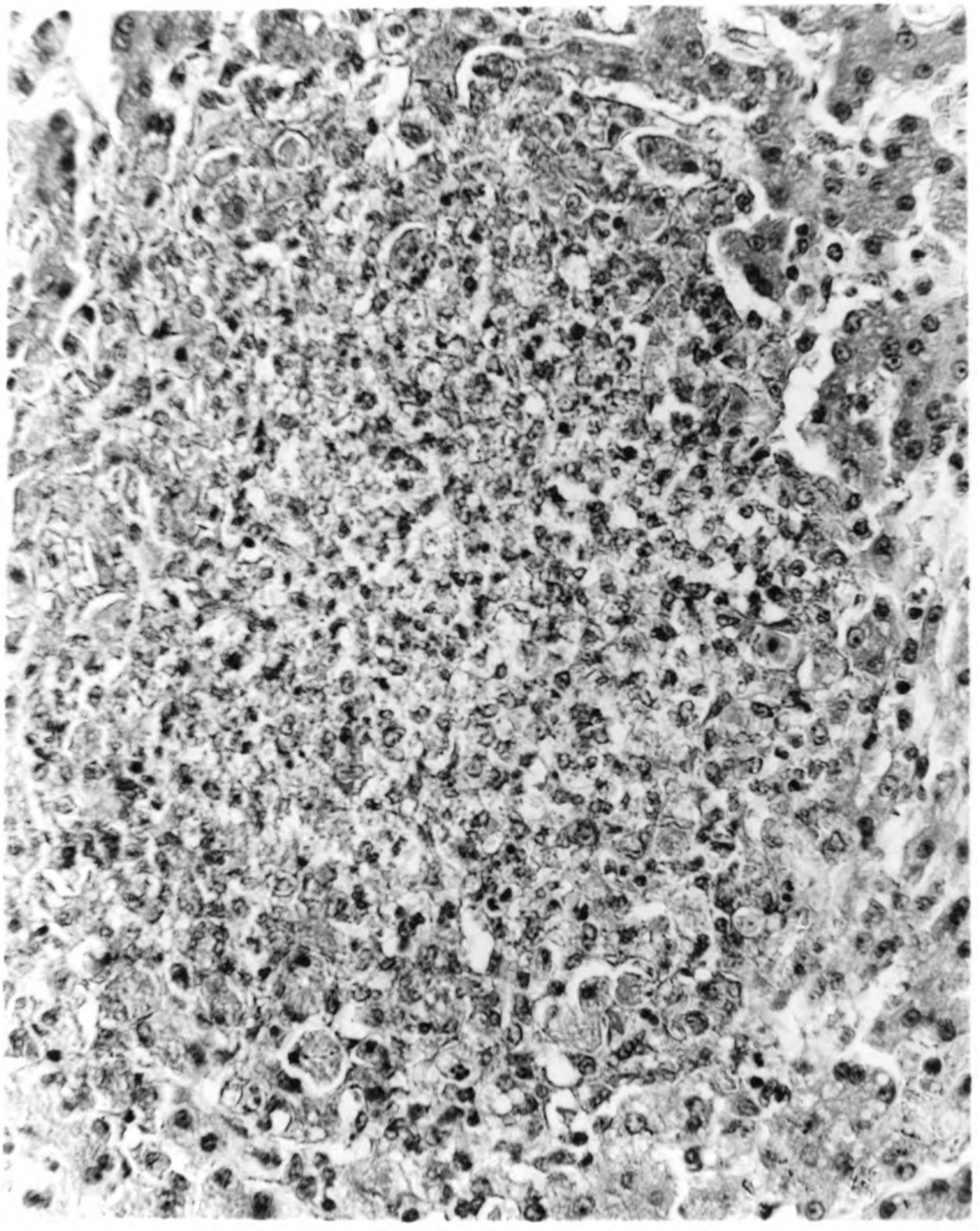
Tonsil - depletion of lymphoid elements; replacement of germinal centers by epithelioid cells.

Bronchial lymph node - depletion of mature lymphoid elements; proliferation of reticulo-endothelial elements.

Lung - diffuse, acute, purulent bronchopneumonia.

Spleen - lymphocytic depletion.

Fig. 5. Liver Dog 6337-9. Canine Distemper Complex.
Focal necrosis with infiltrating lymphocytes, macrophages, and neutrophiles. X400.



Suprapharyngeal lymph node - depletion of lymphocytes, mild hemorrhage and edema in medulla; proliferation of reticuloendothelial elements.

Submandibular lymph node - medullary hemorrhage and edema; loss of follicular architecture and replacement of several germinal centers by epithelioid cells.

Nasal passage - mild acute purulent rhinitis.

DOG 6337-10

Black and Brown Mongrel Male BSP retention: 5.9%

Clinical:

Temperature, 103.1°F; mucopurulent ocular and nasal discharge; tonsils congested; coughing; diarrhea with tan, mucoid feces.

Gross Pathology:

Dried exudate at medial canthi; mucopurulent discharge from external nares; heart pale and flabby; multiple, small, white foci, both subcapsular, and deep in the cortex of the kidneys; heavy infestation with ascarids and tapeworms, and a few whipworms; pancreas slightly enlarged with gelatinous, depressed, grayish areas; liver enlarged, diffusely mottled with yellow, red and tan areas - lobules distinct throughout with circular area 1-1/2 cm. in diameter, yellow in color, granular and friable, on the parietal surface of the right central lobe near the hilus of the diaphragm and a lesion similar but approximately .5 cm. in diameter on the parietal surface of the the left lateral lobe; wall of gall bladder slightly thickened and edematous; hepatic lymph node congested, enlarged and edematous.

Histopathology:

Liver - frequent rhexis of the central veins with mild hemorrhage

and congestion concomitantlywith hypertrophy of the venous endothelium and degeneration of the adjacent hepatic cells; multiple small foci of proliferating endothelial cells, fibroblasts and infrequent neutrophiles or eosinophiles; several sections having three to four larval granulomas, one containing an entrapped larva. Approximately 1/2 of section areas involved.

Urocyst - intranuclear inclusion bodies in epithelial cells.

Pancreas - diffuse, subacute pancreatitis.

Kidney - two larval granulomas in cortex; edema of the renal crest and frequent cellular casts in lumens of collecting tubules in this area.

Heart - single larval granuloma in myocardium.

Nasal passage - acute rhinitis.

Suprapharyngeal lumph node - depletion of lymphocytes; sinuses packed with epithelioid cells; germinal centers replaced by epithelioid cells.

Cholecyst - edema of the wall.

Spleen - acute splenitis; lymphoid hyperplasia and depletion of mature cells.

Tonsil - depletion of lymphoid elements; many germinal centers replaced by epithelioid cells.

Hepatic lymph node - essentially same as tonsil; sinuses edematous and contain epithelioid cells in moderately large numbers.

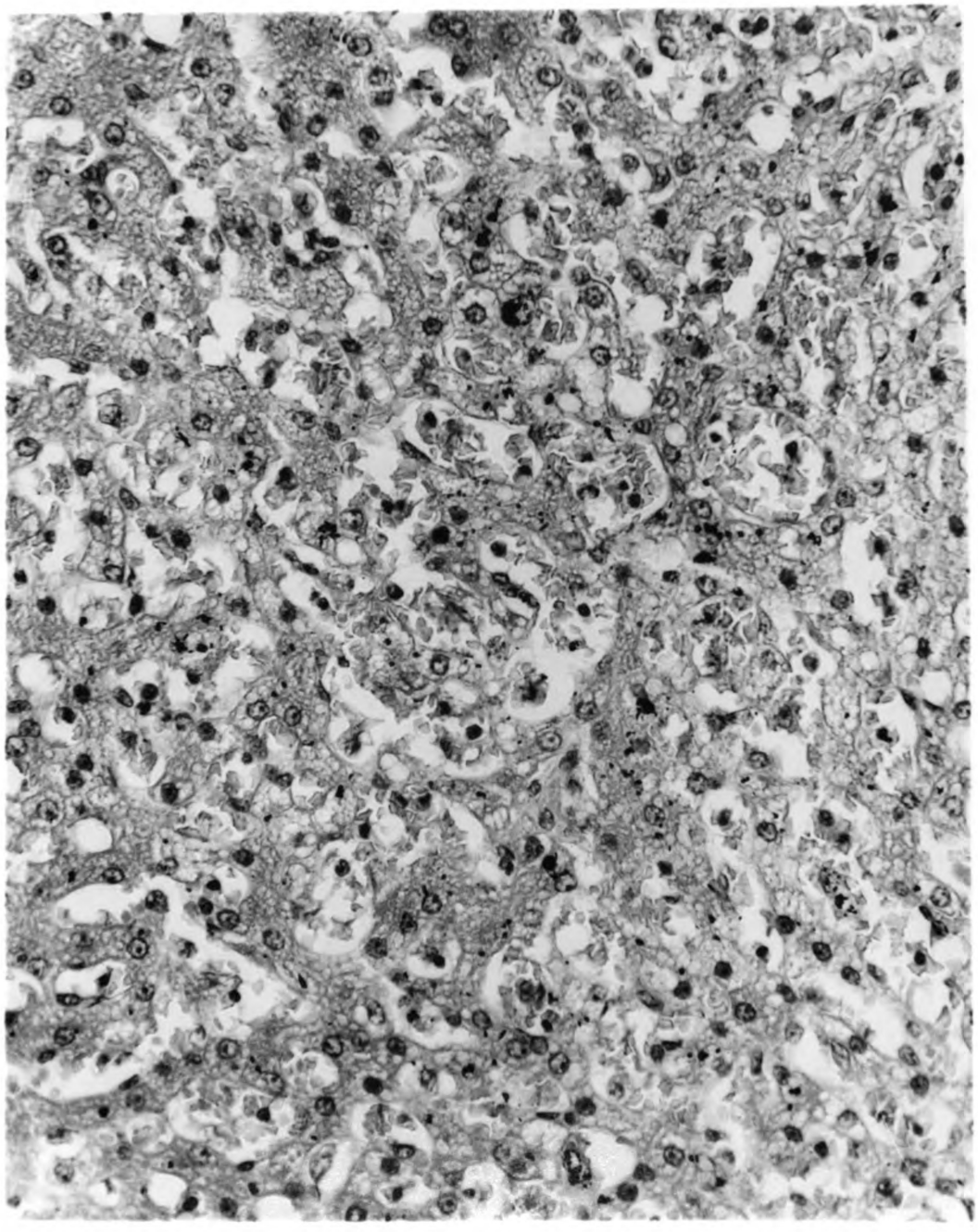
Membrana nictitans - subacute conjunctivitis.

Lung - early focal acute pneumonia.

DOG 6338-a

Beagle Male BSP retention: 2.2%

Fig. 6. Liver. Dog 6337-10. Canine Distemper Complex.
Rhexis of the central vein and centrolobular congestion; adjacent hepatic cord cells are atrophic and vacuolated. X400.



Clinical:

Temperature, 102.4°F; mucopurulent ocular and nasal discharge; soft, painful cough; increased vesicular murmur and areas of dullness on auscultation; diarrhea; depression; sick for one week.

Gross Pathology:

Purulent discharge in nasal cavity; purulent material expressed from bronchi; bilateral red and gray pulmonary hepatization; apical and cardiac lobes adherent; bronchial lymph nodes enlarged and congested; pancreatic lymph node hemorrhagic; gastrointestinal tract empty except for a few small roundworms in small intestine.

Histopathology:

Liver - randomly distributed, relatively few, very fine nidi of predominantly neutrophiles but occasionally lumphocytes and fibroblasts and, in several areas, perivascular cuffing with neutrophiles; one large focus of periductal infiltration with eosinophiles, lymphocytes, a few macrophages, and fibroblastic proliferation.

Lung - diffuse, acute bronchopneumonia and pleuritis.

Urocyst - edema and congestion of the submucous layer.

Membrana nictitans - subacute conjunctivitis.

Spleen - germinal centers frequently replaced by epithelioid cells; a focus of capsular hemorrhage.

Lymph nodes - depletion of lymphoid elements.

DOG 6338-b

Beagle Male BSP retention: 0.8%

Clinical:

Temperature, 103.4°F; mucopurulent ocular and nasal discharge; sclera injected; left Harderium gland enlarged; increased vesicular

murmur; depressed.

Gross Pathology:

In good flesh; slight yellowish discoloration of liver; Harderian gland of left eye enlarged; patchy pneumonia of both apical lobes, red hepatization involving 2/3 of right cardiac lobe along ventral aspect.

Histopathology:

Liver - each section contained several very small foci of lymphocytes and fibroblasts.

Lung - acute purulent bronchopneumonia.

Kidney - unilateral subacute interstitial nephritis.

DOG 6338-c

Beagle Male BSP retention: 1.5% (slight hemolysis)

Clinical:

Temperature, 103.0°F; tonsils congested but not appreciably enlarged; thick mucopurulent ocular and nasal discharges; slightly increased pulmonary lung sounds; sclera moderately injected.

Gross Pathology:

In good flesh; small patches of pulmonary consolidation, ventral areas of both apical and cardiac lobes; gastrointestinal tract empty.

Histopathology:

Liver - slight dilatation of the sinuoids at central vein with very small, scattered foci of lymphocytes, few neutrophiles and an occasional plasma cell or fibroblast.

Stomach - intranuclear inclusion bodies in epithelial cells.

Eyelid - mild subacute conjunctivitis.

Nasal passage - mild acute rhinitis.

Membrana nictitans - mild subacute conjunctivitis.

Tonsil - mild acute tonsillitis.

Spleen - hemorrhage in the germinal centers of the splenic follicles.

Lymph node - mild lymphocytic depletion and moderate amounts of pigment.

DOG 6344-1

Beagle Female BSP retention: 1.8%

Clinical:

Temperature, 101.1°F; very emaciated; anorexia; sclera injected; mild keratitis with slight serous ocular discharge from right eye; mucopurulent nasal discharge; pulmonary sounds indefinite.

Gross Pathology:

Body fat depleted; subcapsular, minute white foci in the cortices of the kidneys; partial mid-lobular consolidation of right apical and cardiac lobes of the lung; slight yellowish discoloration of liver; linear, ecchymotic, multiple hemorrhages in the urocyst; heavy whipworm infestation.

Histopathology:

Liver - no significant lesions.

Kidney - single larval granuloma.

Lung - diffuse, acute bronchopneumonia; one bone spicule deep in lung parenchyma (heterotopic osteogenesis).

Urocyst - early acute cystitis.

Bronchial lymph node - cortical atrophy; abundant pigment.

Suprapharyngeal lymph node - abundant pigment both free and phagocytized.

Tonsil - mild acute tonsillitis.

Spleen - mild acute splenitis; abundant pigment.

DOG 6344-2

Springer Cross Male BSP retention: 0.8%

Clinical:

Temperature, 102.5°F; sclera moderately injected; mucopurulent nasal discharge; induced cough; bilateral increased vesicular murmur and area of dullness over right lung.

Gross Pathology:

In good flesh; pancreas congested; few roundworms in intestine; cryptorchidism, right side.

Histopathology:

Liver - Kupffer cells frequently pigment-laden.

Pancreas - venous congestion.

Spleen - marked lymphocytic depletion; infiltration of red pulp with neutrophiles.

Testis - atrophy and tubular degeneration.

Tonsil - moderate lymphocytic depletion.

Prostate - mild subacute interstitial prostatitis.

Lung - interalveolar congestion; small foci of subacute pneumonia confined to perivascular areas.

DOG 6345-3

Black, Long Haired Mongrel Male BSP retention: 4.0%

Clinical:

Temperature, 103.4°F; slight mucopurulent ocular discharge; profuse mucopurulent nasal discharge.

Gross Pathology:

Two small (3 mm) subcapsular plum-colored areas in the cortex of left kidney with one focus extending into medulla; several slightly raised plum-colored areas in diaphragmatic lobes of lungs; testes and prostate extremely small for size of dog; moderate whipworms infestation; pituitary slightly enlarged and congested.

Histopathology:

Liver - small, rather widely separated, lesions consisting of centers of necrosis or degeneration surrounded by slight hemorrhage, fibrin, loosely arranged lymphocytes, neutrophiles, and a few macrophages; centrolobularly, moderate dilatation of sinusoids; frequent individual neutrophiles occupying sinusoids throughout the section and occasional mild perivascular cuffing with these cells; approximately 1/33 of section involved.

Spleen - moderate lymphocytic depletion; acute splenitis.

Mesenteric lymph node - abundant pigment; germinal centers indistinct; sinuses filled with macrophages.

Prostate - juvenile.

Testis - juvenile.

Kidney - bilateral mild acute pyelitis; focal subacute interstitial nephritis, left kidney.

Lung - congestion and hemorrhage.

Pituitary - venous congestion of pars anterior.

DOG 6345-4

Beagle Cross Male BSP retention: 1.5%

Clinical:

Temperature, 103.4°F; mucopurulent nasal and ocular discharge; sclera injected; induced cough.

Gross Pathology:

Lymph nodes at thoracic inlet enlarged and congested; two adult D. immitis in right ventricle of heart.

Histopathology:

Liver - individual neutrophiles in the sinusoids; mild generalized cloudy swelling; scattered Kupffer cells pigment-laden.

Kidneys - bilateral, moderate subacute pyelitis.

Prostate - mild, focal subacute interstitial prostatitis.

Spleen - abundant pigment, free and phagocytized.

Submandibular lymph node - lymphocytic depletion; follicles atropic; medullary hemorrhage.

Tonsil - lymphocytic depletion.

Suprapharyngeal lymph node - lymphocytic depletion.

DOG 6345-5

Black Cocker Male BSP retention: 0.8%

Clinical:

Temperature, 102.4°F; emaciated; mucopurulent ocular and nasal discharge; left tonsil swollen and congested; induced cough; dull areas on auscultation of lungs.

Gross Pathology:

Few scattered hemorrhages in cecal mucosa.

Histopathology:

Liver - Kupffer cells frequently pigment-laden.

Kidney - two linear foci of subacute interstitial nephritis.

Lung - healing pneumonia (healing by resolution).

Testicle - early tubular degeneration.

Eyelid - focal subacute blepharitis.

Urocyst - mild congestion of the submucous layer.

Membrana nictitans - mild subacute conjunctivitis.

DOG 6443-6

Beagle Male BSP retention: 2.2%

Clinical:

Temperature, 103.0°F; congested sclera; abundant mucopurulent nasal discharge; feces semisolid; harsh rales on auscultation; soft, moist cough when excited.

Gross Pathology:

Nasal mucosa markedly congested with adherent thick mucopurulent exudate; thick mucopurulent exudate in trachea and larynx; right bronchial lymph nodes slightly enlarged, rough with red mottling; liver pale with yellowish cast; cholecyst frosty and wall slightly thickened; hepatic lymph node irregularly congested; moderate roundworm infestation; multiple, elevated hemorrhages ranging from petechial to ecchymotic in mucosa of urocyst.

Histopathology:

Liver - multiple, small, randomly distributed, circumscribed lesions of larval migrans occuring as practically pure granulomas with infrequent eosinophiles or as mixed granulomas with abundant eosinophiles; approximately 1/50 of sections involved.

Kidneys - focal chronic interstitial nephritis, left kidney; focal subacute interstitial nephritis, right kidney.

Lymph nodes - a) multiple larval granulomas (several larvae in sections); lymphocytic depletion; b) lymphocytic depletion and edema; c) lymphocytic depletion and edema; c) lymphocytic depletion and medullary hemorrhage; multiple larval granulomas.

Lung - scattered areas of subacute interstitial pneumonitis with inclusion bodies in septal cells.

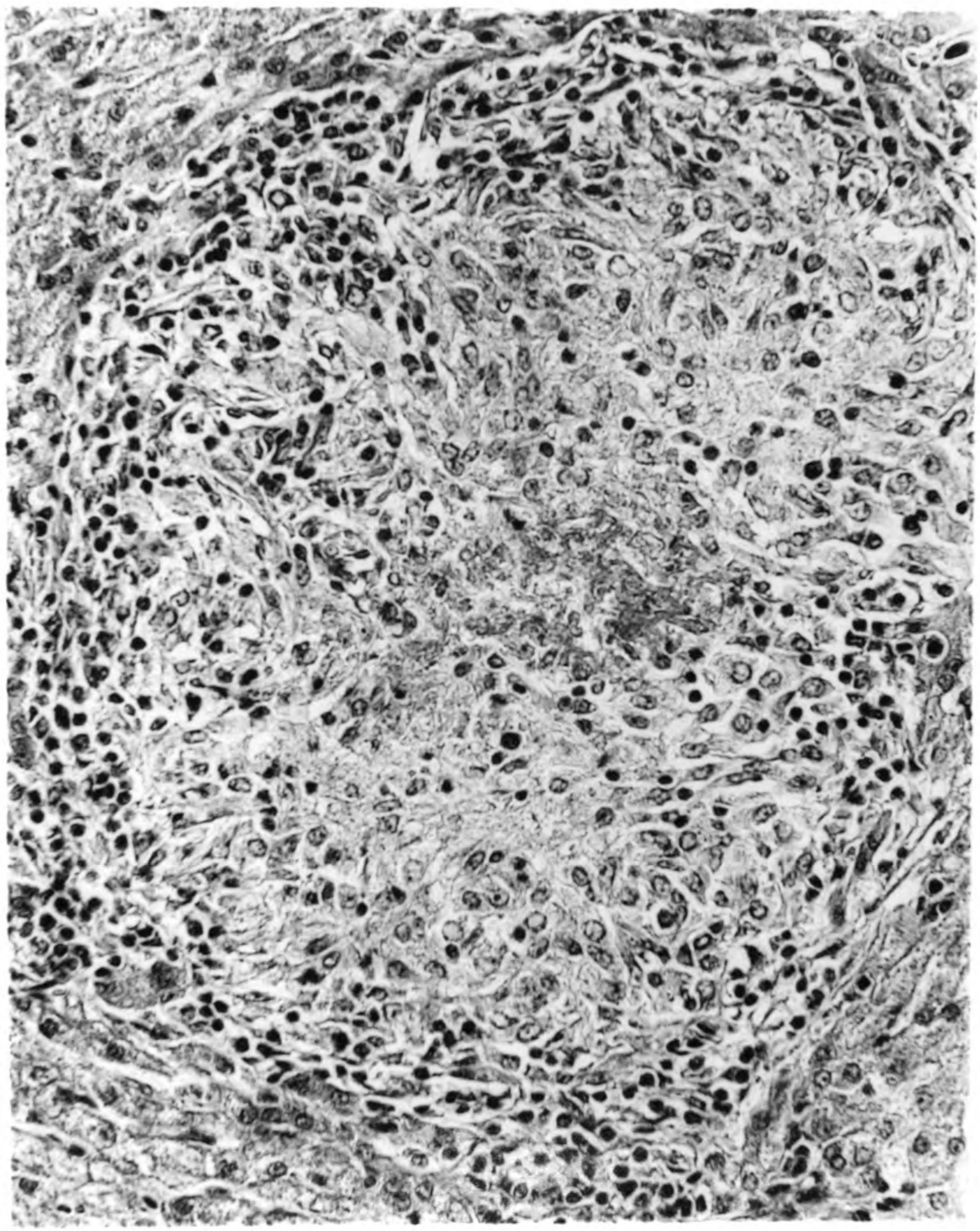
Nasal mucosa - acute purulent rhinitis.

Urocyst - mild active congestion.

Trachea - eosinophilic intranuclear inclusion bodies.

Spleen - marked lymphocytic depletion and marked reticulo-endothelial proliferation.

Fig. 7. Liver. Dog 6443-6. Canine Distemper Complex.
Larval migrans granuloma without eosinophilic infiltration.
X400.



APPENDIX 5

EXPERIMENTAL INFECTIOUS CANINE HEPATITIS DOGS

DOG 6492

Shepherd Cross Female BSP retention: 5.9%

Gross Pathology:

Multiple ecchymotic hemorrhages in submaxillary lymph nodes; suprapharyngeal lymph nodes slightly enlarged and edematous with clusters of petechiae near the anterior pole of right node; slight yellowish discoloration of heart; liver light reddish brown in color with large, irregularly distributed, yellowish areas - on incision, yellowish color with regularly distributed, very fine, yellowish-white foci throughout parenchyma; gall bladder distended and wall edematous; mild roundworm infestation; pancreas reddish brown in color.

Histopathology:

Liver - infrequent intranuclear salt inclusions; in several sections, change in stain affinity of hepatic cell nucleoli from basophilic to acidophilic or polychromatic resulting in a mauve coloration; widely scattered, very fine nidi of lymphocytes with few macrophages or larger foci of fibroblasts and fibrin, located peripherolobularly; centrolobular fatty metamorphosis involving approximately 1/3 of the lobule.

Heart - diffuse fatty metamorphosis.

Kidney - cloudy swelling of thick part of descending loops of Henle.

Suprapharyngeal lymph node - mild acute lymphadenitis.

Submandibular lymph node - multiple small hemorrhages in cortex.

DOG 6681

Collie Cross Male BSP retention: 4.3%

Gross Pathology:

Slight yellowish discoloration to liver; cholecyst enlarged from bile retention; spleen small and pale.

Histopathology:

Liver - few and widely scattered, small foci of neutrophiles, a few lymphocytes and several proliferating Kupffer cells; diffuse cloudy swelling; dilatation of sinusoids at the central vein with cytoplasm of adjacent hepatic cells granular and condensed; lesion that of very mild acute hepatitis.

Cholecyst - edema of submucosal layer.

Tonsil - lymphocytic depletion.

Spleen - mild acute splenitis.

DOG 6760

Tan, White, and Black Mongrel Female BSP retention: 0.8%

Gross Pathology:

Emaciated; left cornea opaque; blood bright red, thin and watery; all visceral lymph nodes enlarged; liver slightly pale; excessive fluid in abdominal cavity; moderate infestation with roundworms and hookworms; spleen small and pale.

Histopathology:

Liver - essentially normal.

Eye - iridocyclitis and edema of cornea without vascularization.

Lymph node - slight lymphocytic depletion and increased macrophages.

DOG 6761

Retriever Cross Male BSP retention: 0.8%

Gross Pathology:

Superficial inguinal lymph nodes enlarged with fleshy streaks within and pigment at corticomedullary junction; bronchial lymph nodes congested; longitudinal tract of edema and hepatization on dorsal aspect of left diaphragmatic lobe of lung; mild hookworm infestation; cholecyst pale and dull with very light-colored bile; multiple, widely dispersed, lesions occurring either as small circumscribed hemorrhages flush with the surface or as very small, white foci with a hemorrhagic border in both kidneys - on incision, white glistening streaks primarily in cortex but extending also into cortico-medullary tissue.

Histopathology:

Liver - essentially normal.

Kidneys - subacute interstitial nephritis and descending subacute pyelonephritis.

Pancreas - single sclerotic lobule with scattered areas of subacute (few areas of acute) interstitial pancreatitis.

Prostate - juvenile.

Cholecyst - mild subacute cholecystitis.

Testis - prepubescent.

Lung - two spherical cysts just beneath the visceral pleura; alveolar interstitium thickened by cellular infiltration consisting of lymphocytes primarily but with abundant proliferating endothelial cells, some fibroblasts and neutrophiles; mild generalized congestion.

DOG 6762

Black Mongrel Male BSP retention: 0.8%

Gross Pathology:

Thick, mucopurulent material in the gastro-intestinal tract but most abundant in the pylorus, duodenum, and jejunum; one roundworm in intestine; several subcapsular, white, glistening foci in liver; several white, glistening foci in the cortex of both kidneys.

Histopathology:

Liver - section 1: centrolobularly, tinctorial change in which hepatic cells stain lightly and cytoplasm more granular than at periphery; sinusoids dilated centrolobularly; widely scattered but relatively numerous small foci either of lymphocytes and proliferating endothelial cells with granular acidophilic material or areas of proliferating fibroblasts suggesting replacement fibrosis in the peripherolobular position; section 2: single large perivenous lesions consisting of eosinophiles, lymphocytes, proliferating endothelial cells, fibroblasts and mild edema, otherwise essentially the same as above; section 3: two relatively large foci of lymphocytes, endothelial cells and fibroblasts, otherwise essentially the same as section 1.

Spleen - depletion of lymphocytes of the follicles but very prominent germinal centers indicating reactive hyperplasia.

Prostate - several circumscribed accumulations of lymphocytes.

DOG 6697

Beagle Cross Female BSP retention: 12.0%

Gross Pathology:

Emaciated; purulent ocular discharge; all visceral and cervical lymph nodes enlarged and edematous; massive edema at thoracic inlet; liver pale with irregular yellowish mottling; mild infesta-

tion with roundworms and tapeworms; spleen pale and slightly enlarged; tissues pale.

Histopathology:

Liver - numerous, diffusely distributed, small, necrotic foci with infiltration of neutrophiles and proliferating endothelial cells; marked perivascular infiltration with neutrophiles and lymphocytes; hepatic cells slightly swollen.

Spleen - marked lymphocytic depletion, increase in reticulum cells and mild neutrophilic infiltration.

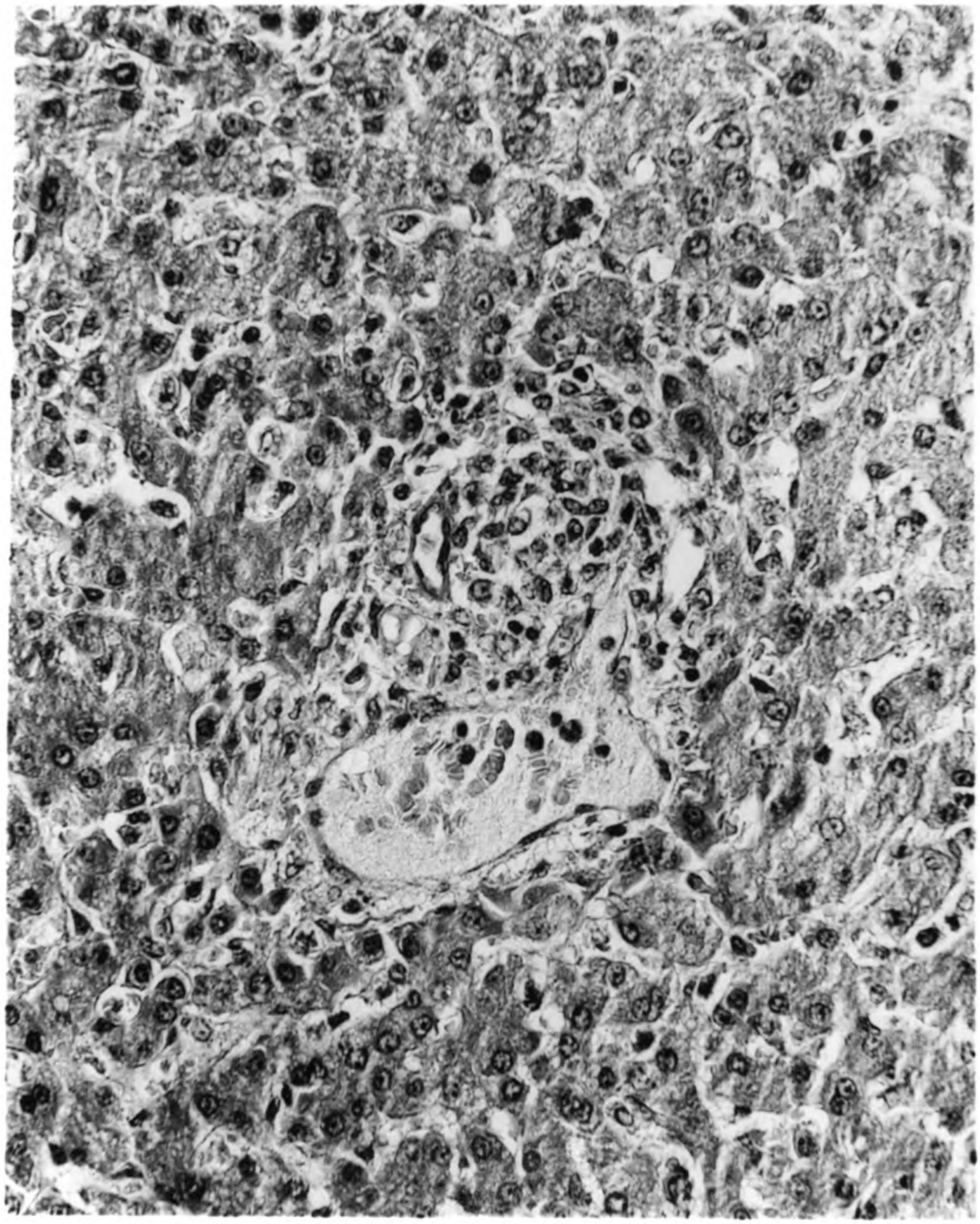
Lung - early bronchiolitis.

Lymph nodes - marked lymphocytic depletion; marked increase in epithelioid cells almost completely obliterating the sinuses.

Lymph node - serous lymphadenitis.

Stomach and urocyst - intranuclear inclusion bodies.

Fig. 8. Liver. Dog 6697. Experimental Canine Infectious Hepatitis. Perivascular lymphocytic infiltration. X400.



APPENDIX 6

PYRETIC DOGS

DOG 6446-1

Black Mongrel Female BSP retention: 0.8%

Body temperature: 103.9°F.

Gross Pathology:

Small intestine congested, especially the duodenum; severe tapeworm infestation; single, isolated, mesenteric lymph node congested.

Histopathology:

Liver - two small larval granulomas; moderate amount of hematogenoid pigment, randomly distributed; lesions involved approximately 1/100 of the area in the sections.

Lung - early focal bronchopneumonia.

DOG 6446-2

Retriever Cross Female BSP retention: 0.8%

Body temperature: 105.6°F.

Gross Pathology:

Right tonsil enlarged and congested with proliferation of lymphoid tissue posterior and downward in confluency with the right suprapharyngeal lymph node which was enlarged to three times its normal size - dorsal surface hemorrhagic or congested entire length of node; mild infestation with tapeworms and roundworms; Peyer's patches prominent; surface of spleen rough.

Histopathology:

Liver - essentially normal.

Tonsil - diffuse suppurative tonsillitis.

Suprapharyngeal lymph node - marked lymphoid hyperplasia and reticu-

lo-endothelial proliferation.

Spleen - lymphocytic depletion but with hyperplastic germinal centers of Malpighian corpuscles.

DOG 6446-3

Beagle Cross Female BSP retention: 0.8%

Body temperature: 105.1°F.

Gross Pathology:

Suprapharyngeal lymph node enlarged and with fine hemorrhages subcapsularly; ileum congested; heavy infestation with hookworms and tapeworms.

Histopathology:

Liver - essentially normal.

Ileum - mild congestion of the mucosa.

Suprapharyngeal lymph node - small, focal hemorrhages in cortex.

Spleen - mild congestion.

DOG 6446-4

Terrier Cross Female BSP retention: 0.8%

Body temperature: 102.8°F.

Gross Pathology:

Suprapharyngeal lymph nodes congested and containing multiple small hemorrhages; mesenteric lymph nodes congested centrally; mild infestation with tapeworms and whipworms.

Histopathology:

Liver - essentially normal.

Suprapharyngeal lymph node - multiple, small focal hemorrhages in cortex and medulla; marked lymphoid hyperplasia.

Mandibular lymph node - mild medullary hemorrhage.

Spleen - marked lymphocytic depletion.

DOG 6446-5

Collie Cross Male BSP retention: 0.8%

Body temperature: 104.6°F.

Gross Pathology:

No significant lesions; essentially normal grossly.

Histopathology:

Liver - slightly granular cytoplasm.

Testis - prepubescent.

Spleen - marked lymphocytic depletion.

Urocyst - edema of submucous layer.