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UNIVERSITY OF LOUISVILLE

THE EFFECT OF SPLENECTOMY ON THE DIFFERENTIAL
CELL COUNT OF THE ANTERIOR PITUITARY IN
RELATION TO POST-SPLENECTOMY CHANGES IN THE
BLOOD PICTURE OF THE DOG

A Dissertation

Submitted to the Faculty

Of the Graduate School of the University of Louisville

In Partial Fulfillment of the

Requirements for the Degree

Of Master of Science

Department of Physiology and Pharmacology

By

Herbert J. Brinker

1937

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Herbert J. Brinker

I. INTRODUCTION.

Within the past two decades the advances of experimental physiology have altered concepts in many phases of medicine. This is especially true of endocrinology. Apparently there are few bodily functions that are not influenced by the endocrine system.

The hematopoietic system is one of the subjects recently brought under investigation. A number of reports of both experimental and clinical observations have been published describing the relation of various endocrine organs to the blood forming tissues.

Schulhof and Matthies (1) after observing polycythemia in epidemic encephalitis, demonstrated long standing polycythemia in rabbits after destructive changes in certain brain areas were induced. They maintained, however, that an intervening endocrine mechanism was not impossible.

After studying the course of eighty patients suffering with various endocrine disturbances, such as, myxoedema, exophthalmic goitre, pituitary tumors, acromegaly and pituitary dystrophy, Moldowsky (2) maintained that endocrine disturbances can produce stimulation of the bone marrow. He also believed that a constant supply of hormone or hormones tends to maintain the normal blood picture. He considered this a function of the entire endocrine system rather than the special function of any particular gland.

Sharpe and Bisgard (3) observed from the study of four totally thyroidectomized rabbits and four control rabbits that a moderate and persistent anemia developed after the removal of the thyroid. The anemia was of the macrocytic type and was accompanied by a slight decrease in leukocytes with no significant change in the differential count or sedimentation rate.

In his discussion of blood changes in myxoedema Zondek (4) stated that in many patients the R. B. C. and hemoglobin are diminished. Unlike Graves' disease in myxoedema the coagulability of the blood is often increased. Under thyroidin treatment all of these changes generally revert to the normal. MacKenzie (5) reported three patients with hypothyroidism in whom an anemia was the most conspicuous clinical feature. Treatment with thyroid gland was followed in one case by prompt and complete recovery and in the other two by marked improvement. Means, Castle et al. (6) stated that myxoedema may resemble pernicious anemia and pernicious anemia may resemble myxoedema. The two diseases may coexist. They said this knowledge is important to the clinician for it seems certain that some patients require treatment with both liver and thyroid.

The clinical observation of the association of diabetes mellitus with pernicious anemia is of interest. Watson (7) in 1933 pointed out that the report of the association of these diseases was published in 1910. Since then 79 cases have been reported. Recently Jolliffe et al. (8) reported additional cases.

In the literature are numerous references to the relation of the suprarenal cortex to hematopoiesis. Zucker (9) reported a case of tumor of the suprarenal cortex in a woman, 35 years old, with polycythemia. Necropsy revealed a carcinoma of the left suprarenal cortex, pulmonary metastases, an enlarged spleen and an atrophic right suprarenal gland. The thyroid and pituitary showed no changes on gross inspection.

Huth (10) gave suprarenal cortex extract to patients suffering from various diseases, seventeen of twenty-six cases showed an increase in erythrocytes.

In recent years there have been a number of reports indicating a relationship between the hypophysis and hematopoiesis. Thus, in a review of the literature on the subject of pituitary relations to erythropoiesis, Moehlig and Bates (11) stated that, "Recently Houssay, Royer and Orias found that in sixteen hypophysectomized rabbits the average hemoglobin content in 100 c.c. of blood was 11.56 Gm., whereas twelve normal rabbits had an average hemoglobin content of 13.6 Gm. The R. B. C. were also diminished in the hypophysectomized animals, but the authors called attention to the marked variation in the R. B. C. count of normal animals. Collin and Baudot, from their work on the guinea-pig, concluded that the embryonic pituitary has a hematopoietic function. Watrin, from his studies, believed that there are erythropoietic centers in the pituitary of the pregnant guinea-pig."

An interesting relation between the posterior lobe of the

pituitary gland and the blood picture was postulated by Dodds, Hills, et al. (12). They reported that when rabbits were injected subcutaneously with a posterior lobe extract they developed a severe anemia by the fifth day. The hemoglobin was also reduced but not so greatly. A marked reticulocytosis occurred about the fifth or sixth day. Necropsy of the animals revealed hemorrhagic infarction of the spleen and evidence of stimulation of the blood destroying system. The intestines were full of bile. They concluded that the anemia may possibly be due to increased blood destruction.

However, Gilman and Goodman (13) considered this work of Dodds et al., and in view of the antidiuretic action of pituitrin and the high water content of rabbit diets thought the anemia might well be due to serum osmotic changes resulting from water retention, rather than a "hemoclastic principle of the posterior pituitary." They studied 15 rabbits injected with posterior pituitary extract in the dosage used by Dodds. Their results showed an evident anemia together with a marked decrease in urinary output, a definite blood dilution, an abnormally low level of osmotically active substances in the blood serum and alterations in the in vitro fragility of the R. B. C. They concluded that there was a definite parallelism between the decrease in serum osmotic pressure and the R. B. C. count. However, the plasma dilution due to water retention was far insufficient in itself to account for the marked reduction in R. B. C. They

interpreted the anemia as a hemolytic one due to hypotonic plasma. They plan further work to clear up parts of the subject that are not well understood.

In discussing the effects of various fractions of pituitary extracts Collip (14) stated, "Two possible effects of the adrenotropic principle, apart from the cortical repair induced in hypophysectomized rats, are (a) a slight decrease in the positive potassium balance of normal animals and (b) an effect on the hemogram. Miss Margaret Hill, who has been making a study of the blood picture as influenced by pituitary extracts, has observed a very definite increase in the reticulocytes when large doses of adrenotropic extracts have been injected daily over a period of weeks. The reticulocyte response appears to follow a definite curve. The significance of this response is not clear."

In studying the relation of the hypophysis to hematopoiesis, Meyer, Stewart et al. (15) used the well known fact that in man and animals, under a condition of reduced oxygen tension, there occurs an increase in hemoglobin, R. B. C. and reticulocytes in the blood with hyp^{er}plasia of the bone marrow. If the hypophysis influences hematopoiesis then hypophysectomized animals might show a different response to reduction of oxygen tension than normal animals. Accordingly they studied groups of rats. In the animals placed in the respiratory chamber 8 to 9 days after hypophysectomy they noted an increase in hemoglobin and R. B. C. similar to normal controls but unlike the

normal they showed a decrease in reticulocytes and a weight loss while in the chamber. In another group placed in the chamber 25 to 32 days after hypophysectomy there was no increase in hemoglobin or R. B. C. and there occurred a marked reduction in reticulocytes and a loss of weight. Examination of the bone marrow of the hypophysectomized rats revealed a lack of the pronounced hyperplasia shown by the controls. They concluded that the results are significant but may not be due to a specific hormone, but to an indirect action on some other organ or to the general derangement of metabolism following hypophysectomy.

The relation of the spleen to hematopoiesis and to the endocrine system has been the subject of considerable experimental work and not a little speculation but our knowledge along these lines is still very deficient.

The function of the spleen has always been uncertain. Most authorities today agree that it acts as a physiologic reservoir for R. B. C.; that it is one of the centers of R. B. C. destruction; that it probably is an important center for thrombocyte destruction. Other functions that are not so definite are: its role in the formation of antibodies and immune substances; its role in the metabolism of purine bodies; its function as an endocrine gland.

In clinical medicine splenic involvement is found in many pathologic states of the reticulo-endothelial system. Varying degrees of splenic enlargement usually accompany the leukemias,

Hodgkin's disease, pernicious anemia, the common chronic anemias and the rarer types of blood dyscrasias, such as, sickle-cell anemia. In other disturbances of the reticulo-endothelial system the spleen is one of the primary sites of pathology, as in splenic anemia, hemolytic jaundice, Gaucher's and Christian's disease. The malignant influence of the spleen in certain of these diseases is demonstrated by the favorable effect of splenectomy.

Observations upon splenectomized dogs or human beings have not been very helpful in throwing light upon the functions of the organ.

Krumbhaar (16) has shown that in the normal mammal splenectomy causes a temporary anemia, varying in degree and duration in individuals and species. There is usually a decrease in hemoglobin, leukocytosis, thrombocytosis and reticulocytosis. The anemia has been explained as being due to the loss with the spleen of a property which aids R. B. C. formation, such as, bone marrow stimulation or conservation of the iron of broken down R. B. C. After splenectomy this stimulation is lost until apparently compensated by the remainder of the reticulo-endothelial system. Evidence of increased activity in the liver, lymph nodes and bone marrow after splenectomy has been reported by Krumbhaar and others.

Combs (17) studied human subjects five years after splenectomy and found a fairly normal blood picture. He stated,

however, that the R. B. C. did not vary with exercise and the duration of voluntary apnea was not increased with successive efforts as in normal subjects.

Leake (18) and others described experiments which indicate that a substance of the nature of a hormone can be obtained from the spleen which influences the hematopoietic activity of bone marrow.

Hoenlin and Schliephake (19) injected human subjects with a splenic extract and concluded that the spleen has a regulating influence on the coagulation rate of blood. The erythrocyte and thrombocyte counts generally increased, but the reticulocytes were uninfluenced.

Chahovitch (20) et al. showed that in dogs poisoned with phenylhydrazine the products of digestion of the spleen in vitro have a stimulating effect on bone marrow and aid in the regeneration of blood. In rats suffering from Bartonella anemia similar results were obtained.

These observations suggest that the spleen is concerned with hematopoiesis, through a mechanism as yet unexplained. In view of the foregoing reports linking the pituitary gland and the spleen with the hematopoietic functions of the body, it is interesting to note that there are recent results indicating an interrelationship of the pituitary and spleen.

Smith (21) noted that hypophysectomy in rats is followed by a decrease in the size of the spleen as well as other viscera

and endocrine glands. Daily homeotransplants of the anterior pituitary gland caused a return of the viscera, including the spleen, to normal or nearly normal proportions.

On the other hand Houssay et al. (22) after an investigation on adult dogs and puppies concluded there was no atrophy of the spleen after hypophysectomy.

Perla (23) reported that removal of the hypophysis in adult rats is followed by progressive atrophy of the spleen. After two months the ratio of spleen weight to body weight was one-half normal. Administration of hypophyseal emulsion repaired considerably the atrophy of the spleen. Hypophysectomy completely inhibited regeneration of the splenic tissue after partial splenectomy. Administration of anterior hypophyseal emulsion restored the regenerative capacity of splenic tissue of hypophysectomized rats to normal. Daily administration of anterior hypophyseal emulsion to rats resulted in hypertrophy of the spleen to twice normal size. This increase in size of the spleen was due primarily to marked hyperplasia of the reticular and endothelial cells of the red pulp. The follicles also increased in size, the reticular tissue of the bone marrow was similarly increased. Erythropoiesis seemed unaffected as no significant increase in the circulating blood cells was observed. The spleen stimulating factor was not present in the fraction containing thyrotropic and adrenotropic hormones. It was present in some degree in fractions containing growth and gonadotropic factors.

It was also present in fractions relatively free from growth hormones. Perla concluded that, "It would seem probable that a spleen stimulating factor or hormone in the anterior hypophysis may exist. Though the evidence is suggestive at present it cannot be stated with certainty, that the spleen stimulating factor is separable from the growth and gonadotropic principle."

From this experimental and clinical evidence, strongly suggesting a relation of both the anterior pituitary and the spleen to the blood-forming tissues and also a relation of the anterior pituitary to the spleen, the question arises of a possible splenic influence on the hypophysis which might in some way modify the hypophyseal effect on the hematopoietic system. The reciprocal nature of the relation of the hypophysis to certain endocrine glands is well known. Experimental and clinical literature is rich with references to the secondary changes seen in the hypophysis following removal or primary change in one or more of the endocrine glands, particularly the thyroid, suprarenals and gonads. Zondek (6) pointed out in his discussion of the hypophysis that during pregnancy the pituitary increases in size. This was due not so much to general cellular hyperplasia as to the increase of cells with large nuclei and granulated cytoplasm, the so-called "pregnancy cells." This change occurred in man, and was described in cats, rats, and guinea-pigs but not in rabbits. The typical change of pregnancy was stated to be produced experimentally in the female guinea-pig, but not in the male, by administration of ovarian hormone. Removal of the

ovaries, i.e. castration, was shown to influence the anterior pituitary by Fichera (24). As a rule, the organ increased in size and the acidophiles became more numerous. Berblinger (25) reported an increase of chromophobe and basophilic cells after thyroidectomy. Moehlig (26) and Moehlig and Bates (11) have reported considerable work on hypophyseal-suprarenal relations and concluded that, "Primary disease of the suprarenal cortex results in secondary pituitary changes, thus accounting for many signs and symptoms erroneously ascribed to the suprarenal cortex." We therefore thought that it would be of interest to study the anterior pituitary gland, in dogs, for possible gross or histologic changes following splenectomy. Noting particularly any difference in the anterior lobe findings during the height of the post-splenectomy blood changes, as compared to the anterior pituitary findings in splenectomized dogs, whose blood had returned to normal.

II. MATERIAL AND METHODS.

We used a group of twelve, adult, male, mongrel dogs of various ages, weighing from 8-15 kgm. They were provided separate cages and a diet of commercial dog food and water supplemented with fresh meat and bones once a week.

Blood samples were taken between the hours of 9-11 A. M., from November, 1936 to April, 1937. The blood was obtained by puncture of the saphenous vein and collected in small glass tubes containing dry potassium and ammonium oxalate as an anticoagulant (27). The blood values determined were: the number of red blood cells, white blood cells and platelets; reticulocyte percentage; hemoglobin; and the volume of packed red cells. The blood cell counts followed the usual technic of counting eighty small squares for red cells and four large squares for white cells.* Hemoglobin was determined in a Newcomer hemoglobinometer. The volume of packed red cells was determined by the capillary method of Guest and Siler (28). Platelets and reticulocytes were enumerated after the wet-mount technic of Dameshek (29) with slight modification. Mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were calculated (30). The blood studies were made at weekly or biweekly intervals over a period of ten weeks to es-

*The hemocytometers, red and white cell pipettes were certified by the U. S. Bureau of Standards.

tablish the normal. Six of the dogs were then splenectomized, three dogs underwent dummy or sham splenectomies and the remaining three were left alone to serve as normal controls. Splenectomy was done with ether anaesthesia using morphine and atropine preoperatively.

Blood studies were then carried out at weekly intervals until the estimated maximum post-splenectomy reticulocytosis occurred when the animals of half of the entire group were sacrificed and studies of their hypophyses made. The remaining splenectomized animals were allowed to continue until their reticulocyte percentages returned to normal when they and the rest of the dummy splenectomized and the normal control animals were sacrificed and hypophyseal studies made. The animals were sacrificed with chloroform. At necropsy the dogs of the splenectomy group were examined for accessory spleens, none of which were found. About fifteen minutes post-mortem the hypophyses were weighed and placed in fixing solution. Fixation and staining were carried out after the method of Severinghaus (31). The material was cut in 5 μ sections and a differential cell count done on the anterior lobe after the method of Rasmussen (32) (33).

The Severinghaus technic is a modification and composite of many of the older methods. It was developed to obtain a sharp differentiation of the main cell types as well as a clear separation of the mitochondria from the acidophilic granules. Briefly this method consists in osmic acid fixation, using potassium dichromate as a mordant. Acid fuchsin is used for staining, together with alcoholic methyl green and acid violet. Differentiation with picric acid is combined with the use of phosphomolybdic acid, oil of cloves and the action of the counter stain to obtain proper differentiation of the acid fuchsin.

The sections were cut and differential cell counts made after the method of Rasmussen (32). The paraffin-embedded pituitary gland is oriented as well as possible so that the sections can be cut in a horizontal plane. By determining the vertical diameter of the gland the total number of sections can be estimated. After cutting about one-fourth of the sections at 10 μ , fifteen sections are cut at 5 μ . Cutting is continued at 10 μ until halfway thru the block when about fifteen more sections are cut at 5 μ . When three-fourths thru the block, fifteen more sections at 5 μ are taken. Only the 5 μ sections are used for counting. The most favorable sections from each level are explored systematically under cedar oil immersion, with a mechanical stage. Epithelial cells in every third microscopic field in every third row of fields were counted. Only cells containing nuclei were counted as well as only cells sharply differentiated (a small number of cells were difficult to classify). In this manner about 200 to 300 microscopic fields were studied and a total cell count of 8,000 to 11,000 cells in each hypophysis made.

III. RESULTS

1. Blood Studies.

The results of the blood studies can perhaps be best understood by a consideration of the accompanying Figures 1 to 5 inclusive. The legend with Figure 1 shows that the dogs were divided into three groups: (1) Splenectomized animals, represented by black lines, (2) Dummy operation animals, represented by red lines, (3) Normal control animals, represented by blue lines. These groups were further divided into subgroups: (A) The animals sacrificed at the height of the post-splenectomy reticulocytosis, represented by broken lines, (B) The animals sacrificed after the reticulocyte percentage returned to normal, represented by solid lines. The marker "♣" indicates the time of operation. The splenectomies and dummy-splenectomies were done during the week of January 17-24, 1937. The marker "♠" represents the peak of the reticulocytosis when the dogs of the (A) subgroups were necropsied, and the marker "♣" shows when the (B) subgroups were necropsied.

The curves represent the average values for the animals in the subgroups, except in (2) B, and (3) A, where the subgroups contained only one dog each.

Figures 1, 2 and 3, representing the average values for R. B. C., hemoglobin and R. B. C. volume percent, respectively, show a decrease after splenectomy but as a reduction of about the same magnitude was shown by the control groups, the changes are not significant.

FIG. 1. THE RED BLOOD CELL COUNTS OF SIX SPLENECTOMIZED AND SIX CONTROL DOGS.

GROUPS

1. SPLENECTOMIZED DOGS
2. DUMMY SPLENECTOMIZED DOGS
3. NORMAL CONTROL DOGS

SUBGROUPS

A- NECROPSIED AT HEIGHT OF POST-SPENECTOMY RETICULOCYTOSIS
B- " " END " "

A	-----	3 DOGS
B	—————	3 DOGS
A	-----	2 DOGS
B	—————	1 DOG
A	-----	1 DOG
B	—————	2 DOGS

⬆ WEEK OF OPERATION - ALL DOGS

⬆ DAY OF NECROPSY - SUBGROUP A

⬆ DAY OF NECROPSY - SUBGROUP B

FIG. 2. HEMOGLOBIN VALUES OF SIX SPLENECTOMIZED
AND SIX CONTROL DOGS.

LEGEND AS IN FIG. 1.

FIG. 3. THE RBC VOLUMES PERCENT OF SIX SPLENECTOMIZED AND SIX CONTROL DOGS.

LEGEND AS IN FIG. 1.

FIG. 4. THE RETICULOCYTE PERCENTAGES OF SIX SPLENECTOMIZED AND SIX CONTROL DOGS.

LEGEND AS IN FIG. 1.

FIG. 5. THE PLATELET COUNTS OF SIX SPLENECTOMIZED
AND SIX CONTROL DOGS.

LEGEND AS IN FIG. 1.

Figures 4 and 5 however, representing the course of the reticulocyte percentage and the number of platelets, respectively, show a rather marked increase in value beginning about the third week after splenectomy, reaching a maximum about six weeks after operation, after which the values tend to return to normal. The reticulocyte percentages reached normal figures about nine or ten weeks after operation. The platelets were somewhat above normal at the time of necropsy.

The leukocytes showed a slight increase in value after splenectomy but not beyond the normal variations. The pituitary glands removed at the height of the post-splenectomy reticulocytosis showed no significant difference in weight from the pituitaries removed after the reticulocytes had returned to normal.

2. Hypophyseal Studies.

Table I^{p. 19a} represents the frequency distribution of the percentage of the various types of epithelial cells of the anterior lobe of the pituitaries. The results are arranged under the same groupings and subgroupings that are carried through the Figures for the blood studies.

Analysis of the table suggests the possibility of an increase in chromophobes at the height of the post-splenectomy reticulocytosis with a concurrent decrease in acidophiles. While after the reticulocyte percentage returned to normal there is a suggested decrease in chromophobes with an increase in acidophiles. The basophiles remained uniformly distributed during both periods.

It is to be noted that a fairly wide range of variation for all cell types occurred in the control groups.

IV. DISCUSSION

1. Blood Studies.

The results of blood studies in our animals show that only one of the six splenectomized dogs developed a marked anemia post-operatively. This dog, which belonged to subgroup B, showed a normal range of R. B. C. from 5.63 to 7.96 millions per cmm. before operation. About four weeks after operation this dog's R. B. C. count dropped to 2.21 millions. The count remained in this range for about three weeks after which it slowly returned to normal. A corresponding reduction in hemoglobin and R. B. C. volume percent accompanied the drop in R. B. C. The remaining five splenectomized dogs showed R. B. C. hemoglobin and hematocrit values within the normal range. These findings are not in agreement with those of Krumbhaar (16) who found that 100% of his dogs developed a post-splenectomy anemia. However, as pointed out by him in his review of the literature on the subject, about forty percent of the authors who studied dogs, reported results which showed no marked changes in R. B. C. or hemoglobin, in their investigations. Moreover, Krumbhaar stated that the average drop in R. B. C. in his series was 1.49 millions, the extremes being 0.5 to 3.5 millions. It has recently been shown by investigators (34) (35), that in dogs under normal conditions, the R. B. C. may vary 1.5 millions within a period of seven days and that variations of 1.0 million were not unusual. The normal variations in R. B. C. are more or less closely ac-

accompanied by corresponding changes in hemoglobin and hematocrit values. In describing changes in blood pictures in man or animals the normal range and the normal variation must necessarily be taken into account. Wintrobe (34) reported the following values for normal dogs:

R.B.C.	Extremes	5.3-8.9 millions;	Mean	7.02 $\pm$.03
Hemoglobin	Extremes	12-18 grams;	Mean	14.60 $\pm$.07
R.B.C.Vol. %	Extremes	35-59 percent;	Mean	47.30 $\pm$.24

With the exception of the one dog previously mentioned, our figures for control and operated groups, before and after splenectomy, practically all fall within these ranges.

We found an increase in the percentage of reticulocytes to be one of the marked changes in the post-splenectomy blood picture of dogs. Significant increases occurred in all of the splenectomized animals. Values for the maximum reticulocytoses ranged from 2.0 to 6.2 percent, with an average of 3.6 percent, as compared with a range of from 0 to 0.8 percent for the dummy-splenectomized and normal control dogs. This post-splenectomy reticulocytosis has been previously described by a number of authors (16).

We also found a significant change in the number of platelets in the blood following splenectomy. The increase began about four weeks after operation, reached a maximum about six weeks after operation and then tended to return to normal. The values for the maximum platelet counts in the various splenectomized dogs ranged from 0.65 to 2.45 millions per cmm., with an average of 1.40 millions. In the dummy splenectomized and

normal control dogs the number of platelets ranged from .17 to .88 millions. A similar post-splenectomy thrombocytosis has been previously described by many authors although there is disagreement as to the duration of the change (36) (37).

The increase in platelets does not exactly parallel the changes in the reticulocyte percentage, since the former were still considerably increased after the reticulocytes had reached normal levels. This is interesting in view of Howell and Donahue's recent work (38), in which they concluded that the production of platelets was not an important bone marrow function but that, probably the chief site of production is in the lungs.

2. Hypophyseal Studies.

The percentage distribution table for the cell types of the anterior pituitary suggest an increase in chromophobes with a decrease in acidophiles at the height of the post-splenectomy reticulocytosis. A decrease in the chromophobes with an increase in acidophiles, is suggested, after the reticulocytes have returned to normal. As the table shows, these changes are not striking, but they are sufficiently interesting to make one reluctant to ignore them. Although we have not found any definite evidence that alterations in the differential cell count of the hypophysis occur during the course of the reticulocytosis and thrombocytosis which follow splenectomy, in the dog, it is probably safe to say that these results suggest such a possibility. A curious feature of the distribution table is seen in

TABLE 1. DIFFERENTIAL CELL COUNTS OF THE ANTERIOR PITUITARIES
OF SIX SPLENECTOMIZED AND SIX CONTROL DOGS.

PERCENTAGE OF CHROMOPHOBES	GROUP 1		GROUP 2		GROUP 3	
	SPLENECTOMIZED DOGS		DUMMY SPLENECTOMIZED DOGS		NORMAL CONTROL DOGS	
	SUBGROUP*		SUBGROUP		SUBGROUP	
	A	B	A	B	A	B
	#1,9,10	#4,5,6	#2,7	#8	#13	#11,12
46-47.9		5				
48-49.9		4,6				
50-51.9						12
52-53.9			7			"
54-55.9						
56-57.9				8	13	
58-59.9	10					
60-61.9	1,9					
62-63.9			2			
MEAN %	60.46	47.24	57.46		53.50	
ACIDOPHIKES						
18-20.9	1,10		2			
21-23.9	9		7			
24-26.9				8		
27-29.9						"
30-32.9		6			15	
33-35.9						
36-38.9						12
39-41.9		5				
42-44.9		4				
MEAN %	21.33	38.46	22.66		32.0	
BASOPHIKES						
8-9.9		4				
10-11.9					13	12
12-13.9		5				
14-15.9	9					
16-17.9				8		
18-19.9		6	2			"
20-21.9	1					
22-23.9	10					
24-25.9			7			
MEAN %	19.47	14.26	20.26		13.55	

*SUBGROUP A - DOGS NECROPSIED AT HEIGHT OF POST-SPLENECTOMY RETICULOECYTOSIS
SUBGROUP B - " " " END " " "

the splenectomized group, where the hypophyses removed at the height of the reticulocytosis show chromophobes in the range from 58-62% and acidophiles from 18-24%, representing opposite extremes in their respective distributions. The hypophyses removed after the reticulocytes had returned to normal show an almost complete reversal in these percentages. The chromophobes range from 46-50%, the acidophiles in two dogs from 39-43%, one dog, however, showed only 30%. These results may represent the happenings of chance, then again they may be significant. The number of animals in our group is small and the differential cell count values for the control animals showed considerable variation, which facts make one cautious in interpreting results. Therefore we feel that more work should be done on another and larger group of dogs, before deciding whether these changes are significant or not. Furthermore, certain difficulties arise in carrying out differential cell counts on dogs' hypophyses. A number of the cells that we encountered were not sharply differentiated, seemingly having characteristics of more than one cell type, making its classification uncertain. Just how many such cells were run across is hard to estimate, possibly one percent of the cells encountered. This situation seemed to be present in all of the hypophyses. We attempted to avoid a sizeable error, or at least make our error fairly constant for all the glands by simply not counting those cells that were poorly differentiated. This difficulty may be due to our faulty

technic but it is possible that some of these cells represent transitional states of the three types of epithelial cells, as Susman (39) suggested. Disregarding technical troubles, further difficulty in interpreting the histologic picture of the anterior pituitary is apparent when it is realized that some of the most fundamental points are still a matter for debate. Although almost all authorities agree that there are three main types of epithelial cells in the anterior pituitary, the relation of the various types to each other and the significance of changes in the percentage of the cell types is not clear. This is largely due to the fact that it has not been decided, as yet, whether these types of cells are distinct entities or whether they merely represent different phases of a single type of cell. According to Stewart (40) Blair-Bell (41) Susman (39) and others, the three types of cells represent different stages in the physiologic activity of a single type, while Cushing (42) was the chief exponent of the theory that they each represent a distinct cell type with, apparently, distinct functions.

There has been disagreement as to the normal percentage of the various types of cells and how these percentages vary with age, body weight, height and the like. Some writers describe the normal human, adult anterior pituitary as having acidophils in greatest number, basophils next, with chromophobes fewest of all (41) (43) (44). Others maintain that acidophils make up the great majority of cells with relatively few basophils and chromo-

phobes (45). Rasmussen (32) concluded that in man, chromophobes predominate with acidophils next and basophils fewest. He pointed out the necessity for standardization of the methods of studying the anterior pituitary.

Frey (46) recently reported an interesting study on dogs. He attempted to find a relation between the pituitary gland and the general body shape and size of various breeds of dogs. He studied the smaller breeds, such as, dachshunds, poodles, etc., and the larger breeds, such as, hounds, pointers and the like. His findings led him to believe that the pituitaries of various breeds have characteristic histologic pictures. He stated that the predominant cell in the anterior pituitary of hounds was the basophile while in the smaller breeds acidophils composed the majority. If these findings represent the normal pictures it is obvious that studies on mongrel dogs might well be difficult to evaluate. Frey's data on the relative number of chromophobes, acidophils and basophils in the pituitaries of dogs are qualitative in character. To our knowledge the differential cell counts for the pituitary of the dog presented in this thesis constitute the first recorded quantitative data for this species.

V. SUMMARY

1. Splenectomy produced no significant changes in R. B. C., hemoglobin or hematocrit values of five out of six dogs. The sixth dog showed a post-splenectomy anemia which was most severe at the end of the fourth week after operation and was recovered from at the end of the eighth week.
2. Splenectomy was followed in all dogs by a definite, though temporary, reticulocytosis and thrombocytosis.
3. Differential cell counts of the chromophobes, acidophils and basophils of the anterior pituitaries of six splenectomized dogs, together with three dummy-splenectomized and three normal control dogs, are recorded, to our knowledge, for the first time.
4. Questionable evidence was obtained for an increase in the percentage of chromophobes with a decrease in acidophils, at the height of the post-splenectomy reticulocytosis and for an inverse change in the histologic picture of the anterior pituitary, when the reticulocyte percentage returned to normal. Further work with a larger series of dogs is necessary to rule out or prove the significance of this suggested finding.

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