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The effect of experimental liver damage on the blood picture of the dog.

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THE EFFECT OF EXPERIMENTAL LIVER DAMAGE ON THE BLOOD PICTURE OF THE DOG

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CONTENTS

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INTRODUCTION

Work during the past nine years suggests that various macrocytic hyperchromic anemias, of which pernicious anemia is an example, may result from: 1) the absence of a so-called intrinsic factor, normally contained in the gastric secretion (1,2); 2) the absence of an extrinsic factor associated with the nitrogenous portion of the diet(3); 3) the lack of absorption(4,5,6,7,8) of the product of the interaction(2,9) of the intrinsic and extrinsic factors; 4) the interference with or derangement of the storage mechanism for this principle in the liver(10,11,12,13,14,15). Acute or chronic liver damage as seen clinically may be accompanied by a typical macrocytic hyperchromic anemia presenting in a greater or lesser degree the cytological changes characteristic of pernicious anemia. Further, these patients respond promptly to treatment with liver preparations known to be effective in pernicious anemia. Wintrobe and Shumacker(16), reviewing forty-three cases of hepatic cirrhosis, note a 37% incidence of macrocytic hyperchromic anemia. Van Duyn(17) reports an 18% incidence in twenty-eight cases of hepatic cirrhosis. Malamos (18) reports that in each of twenty-four cases of diffuse liver disease studied, the mean diameter of the erythrocytes was increased by 0.6 - 2.3 micra, together with a widening of the Price-Jones curve.

Experimental corroboration of these clinical findings appears to be wanting. Saito(19), working chiefly with rabbits, found a distinct diminution in erythrocytes and hemoglobin upon excision of the liver, but the anemia produced bore no similarity to any of the macrocytic hyperchromic anemias. Bracaloni and Montanari(20), having subtotally hepatectomized rabbits which subsequently died within five days due to hepatic insufficiency, found an increase in the number of the younger cells of the leukocytic series and an increase in the nucleated and basophilic cells of the erythrocytic series in the circulating blood. They suggested that this observed blood picture was due to the removal of part of the reticulo-endothelial system. Pelligrini(21) reported that following total hepatectomy on frogs kept at a low temperature, there occurred an intense anemia; control operated animals showed a less marked but nevertheless definite anemia. He observed in the circulating blood a significant increase of the immature forms of the erythrocytic series throughout the animals' subsequent life, which usually was 10 to 15 days with an extreme of 24 days. The bone marrow of the hepatectomized frogs showed an active proliferation of the erythrocytic elements accompanied by an arrest of the cyto-evolutive processes at the erythroblastic stage. Bollman and Mann(22,23) made occasional blood counts on their partially hepatectomized dogs and reported the presence of only a mild microcytic hypochromic anemia. About the time of

completion of the work reported in this thesis, Higgins and Stasney(24) reported that an experimental cirrhosis of the liver together with a macrocytic anemia can be obtained in white rats by the inhalation of carbon tetrachloride vapor. The anemia produced is a macrocytic hypochromic anemia, thereby differing significantly from the type of anemia previously cited as associated with certain instances of hepatic cirrhoses in man. The carbon tetrachloride, moreover, affected organs other than the liver.

The foregoing facts suggested a study of the effect of chronic liver damage on the blood picture of the dog. It was hoped that all or a part of the liver damaged animals would show evidences of a macrocytic hyperchromic anemia of such degree as to render them suitable for the bioassay of the antianemic activity of liver extracts. Such an animal would be useful in investigating both abnormal and normal hematopoiesis.

The liver of the dog appears to carry out a function in hematopoiesis similar to that of the liver of man. Ivy, Morgan and Farrel(25) and Strauss and Castle(26) have found that extracts of canine liver were effective in producing remission in cases of pernicious anemia, although the content of the anti-pernicious anemia principle is one-fourth to one-fifth that of hog liver.

Evidence that the dog may develop an anemia which is hematologically identical with the macrocytic hyperchromic anemias of the human is found in the reports of Storti(27)

and of Syderhelm(28). Storti was able to induce the morphological picture of pernicious anemia in two of three dogs by infestation with Dibothriocephalus ranarum Gastalde. Syderhelm, by means of stenosis of the small intestine induced by operation, obtained a blood picture closely simulating that of pernicious anemia.

METHOD:

A search was made for a finely divided substance which upon injection into the portal circulation would produce interference with the capillary blood supply to the liver sufficient to cause atrophy of the lobules of the liver with subsequent cirrhotic changes and diminished liver function. Finely divided carbon particles were found to vary too widely in size and to be quite irregularly shaped. Starch granules from various sources likewise varied too widely in diameter. Tiny glass spheres, had they been available, would have been most suitable. Lycopodium spores which are entirely inert physiologically, were found to have suitable diameters: between 38 to 112 micra with an average of 68 micra; they were sufficiently spherical to block more or less completely the flow of blood in any vessel of corresponding size in which they might lodge.

Preliminary experiments were performed for the purpose of: 1) determining the strength of suspension of spores which could be injected without clogging either the syringe, the

needle, or the vein; 2) determining the maximum tolerated quantity of spores; 3) corroborating the findings of Mall (29) that material injected into the portal vein is evenly distributed to all parts of the liver.

From eight acute experiments on dogs under ether anesthesia, it was observed that the spores were most easily injected into the portal system and carried into the liver when an 18 gauge 1-1/2 inch needle was inserted into a tertiary tributary of the superior mesenteric vein and pushed up inside the vein just past its junction with another to form a secondary tributary, the suspension then being injected at the rate of 10 cc. per minute. Upon withdrawal of the needle, the vein was ligatured at sites proximal and distal to the puncture. Among the various concentrations of spores, with and without various stabilizing agents, it was found that the most suitable was a one per cent suspension of spores in 0.9 per cent sodium chloride solution which, when autoclaved, remained stable during the time required for injection. Microscopic examination of frozen sections from various portions of the livers of these animals revealed a fairly even distribution of spores in the smallest intrahepatic portal radicles. A few spores were observed in the sinusoids and none in the central lobular veins. Doubtless many more spores were present in the sinusoids of these sections, but were rolled out by the microtome knife.

Thereupon, a series of 17 dogs, 10 males and 7 females,

were operated under sterile precautions, injecting the selected concentration of spores in various doses per kilogram. In the female series, it was found that 10 cc. per kg. was fatal in two dogs and that doses of 7.5, 7.5, 7.5, 5.0, and 2.0 cc. per kg. were tolerated. In the male series, 7.5 cc. per kg. was fatal in two dogs and doses of 2.5, 2.5, and 1.5 cc. per kg. were also fatal; doses of 5.0, 5.0, 2.5, 2.0, and 1.5 cc. per kg. were tolerated by other males. Therefore, a dose of 7.5 cc. per kg. for the females and 5.0 cc. per kg. for the males was selected. At these levels in the final experimental series, there was a 43% mortality in seven females and 33% mortality in six males. With two exceptions, those animals which died did so within two hours after completion of the operation. In most instances they appeared to recover partially from the anesthesia and then to become unconscious. Very strong stimuli failed to elicit reflex activity. The tongue and mucous membranes became progressively paler. The respiration frequently took on Cheyne-Stokes or Kussmaul characteristics. The heart beat became progressively weaker and more rapid. Rectal temperatures of 95 degrees F. frequently were recorded at the time of death. This type of death constitutes an acute form of the clinical syndrome observed in occlusive thrombophlebitis of the portal vein(30). Other workers have reported similar results upon experimental restriction of the blood flow in the portal vein(31,32,33).

Autopsy findings indicated a most severe obstruction to blood flow in the tissues drained by the portal vein. The gut from the distal third of the stomach to the middle of the colon was thickened, turgid and of a bluish-purple color; the mucosa was hemorrhagic and the lumen was filled with chocolate colored fluid. The spleen was three to five times larger than normal and oozed blood upon nicking the capsule. The liver was normal in size and consistancy but dark in color. The chambers of the heart contained but small amounts of blood. The lungs, kidneys, suprarenals, and the distal half of the colon were not remarkable. There was never found more than 50 cc. of fluid in the peritoneal cavity. All the autopsy findings, therefore, pointed to a damming back of the blood in the portal vein and its tributaries.

The final experimental series consisted of six adult male and six adult, non-pregnant female dogs. These animals were entirely unselected except that they were objectively healthy. Throughout the experiment, the animals were housed individually in roomy cages and fed on a standard balanced diet of canned dog food with an occasional bone. They obtained exercise while the cages were being cleaned and on their trips to the laboratory table. They were trained to lie quietly on a table and submit to venepuncture and other handling without objective pain or fear.

During a four week control period, the erythrocytes, hemoglobin, hematocrit and leukocytes were determined at weekly

intervals; the reticulocytes were determined semi-weekly; determinations of liver function by the Rose Bengal method and blood chemistry determinations were carried out on alternate weeks. Blood samples from the saphenous vein were drawn in the morning at approximately the same time for each dog in order to rule out diurnal variations as far as possible.

The blood for counting was drawn into a sterile, dry glass syringe containing heparin in quantities sufficient to make a concentration of one mgm. per 4 cc. of blood. At once the blood was thoroughly mixed with the anticoagulant and by means of a capillary pipette a part was placed in duplicate hematocrit tubes having a 3 mm. bore. The remainder was spread in a thick layer on an alcohol cleansed slide from which duplicate red blood cell, single hemoglobin, single white blood cell and single reticulocyte pipettes* were filled; thereupon, dilutions with appropriate fluids were quickly made. After homogeneous mixing with Hayem's fluid, duplicate erythrocyte determinations were made by filling each chamber of a bright line hemacytometer from separate pipettes and counting four hundred small squares of that chamber. The recorded figure was the average of two counts differing less than 200,000. Leukocyte determinations were made by counting four square millimeters in each chamber filled from the same pipette and recording the average figure. Hemoglobin determinations were obtained by the Newcomer technique and instrument.

*Hemacytometer, red and white cell pipettes were certified by the United States Bureau of Standards.

The hematocrit tubes were centrifuged at 2,200 revolutions per minute for forty minutes in a size 2 type B International centrifuge with a working head of 13.5 cm. radius. Preliminary work showed that at this speed complete packing usually had occurred at thirty minutes and always at thirty-five minutes. Readings were made to 0.25 per cent in the separate tubes and averaged. The top of the gray-red layer of white and red cells overlying the pure red-cell column was taken empirically as the line of demarcation. Thus the technique with certain insignificant adaptations follows that outlined by Wintrobe(34), who states(35) that the greatest variation in each of the constants, which can be attributed to the technique alone, can be represented by the mean coefficients of variation plus three times their standard deviations, which in no instance exceeded 2.5%. This value may therefore be considered the maximum variation attributable to technique.

Using the wet-smear technique outlined by Stitt(36), the reticulocytes were recorded in per cent from a count of 1,000 cells of the erythrocytic series. The Rose Bengal function test(37,38) was selected on the basis of accuracy, and simplicity. The technique described by Delprat and Stowe (37), was found to be applicable to the dogs; throughout, the dose of 2.06 mgm. of dye per kg. was used. Determinations of non-protein nitrogen, urea, creatinine, blood sugar, CO_2 combining power of plasma, and the van den Bergh test were made by the technical staff of the Louisville City Hospital

Laboratories.

Of the twelve animals, four males and four females were selected for the injection of lycopodium spores, the remaining four serving as operated control animals. Each animal was operated the day after the fourth count had been made. The dog, having been fasted for 24 hours, received a preoperative injection of 15 mgm. of morphine and 0.6 mgm. of atropine. Throughout the operation, which lasted an average of two hours, the animal was maintained in light surgical anesthesia by ether administered by the drop method. Under strict sterile precautions a high right rectus layered incision was made, the liver palpated and a biopsy of the liver obtained. The small intestine, preferably the ileum, was delivered into warm physiological saline sponges, a suitable vein selected, the spore suspension injected and the vein tied off. The liver was reinspected for evidence of bleeding from the biopsy area or from a possible rupture due to traction. The wound was then closed with silk in four layers. Control animals were subjected to identical manipulations except that 0.9 per cent sodium chloride solution in doses of 7.5 or 5.0 cc. per kg. instead of the spore suspension was injected into the portal circulation.

The blood studies were continued as during the preliminary control period for nine weeks succeeding operation. Subsequently a clinically active preparation of liver extract*

* Supplied through the courtesy of Dr. Guy W. Clark of the Lederle Laboratories, Inc., Pearl River, N. Y.

(3 cc. derived from 100 grams of fresh liver) was injected into the hamstring muscles in doses of 0.2 cc. per kg. daily for five days. Beginning with the third injection of liver extract, semi-weekly samples of blood were obtained and cell counts made. Reticulocyte counts were made daily throughout this period. The alternating sequence of liver function and blood chemistry studies was not disturbed. The period of observation following the liver injections lasted four weeks.

At the end of this period, the animals with three exceptions were sacrificed and autopsied, and specimens of the livers were secured for microscopic examination.

RESULTS:

Data obtained by the described standardized technique from 63 counts on 19 normal dogs which were used in preliminary experiments are summarized in Table I and are submitted for the purpose of comparison with the data of the final experimental group of dogs, with special regard for the range of variation to be expected. The data obtained from the twelve animals of the final experimental group at weekly intervals during the preoperative control period are summarized in Table II. A comparison of Tables I and II indicates that the animals of the final experimental group were hematologically normal during the preoperative control period. In Table II are included the maximum variations noted in any single animal of the final experimental group between two successive sets of

TABLE I

BLOOD VALUES FOR NORMAL DOGS
(63 counts on 19 animals)

	Average	Maximum	Minimum
Erythrocytes per cmm.	6,531,000	8,545,000	5,140,000
Hemoglobin grs. per 100 cc.	13.92	18.1	10.4
Hematocrit per cent cells	45.53	57.00	35.00
Reticulocytes per cent R.B.C.	0.35	1.1	0.0
Leukocytes per cmm.	12,795	23,700	4,500
Mean Corpuscular Volume (cubic micra)	69.73	82.1	57.6
Mean Corpuscular Hemoglobin (gamma gamma)	21.43	26.0	17.6
Mean Corpuscular Hemoglobin Conc. (per cent)	30.75	34.3	27.9

TABLE II

BLOOD VALUES OF DOGS USED IN EXPERIMENT DURING PREOPERATIVE CONTROL PERIOD

	Average	Maximum	Minimum	Maximum variation between 2 successive counts
Erythrocytes per cmm.	6,647,000	8,930,000	5,330,000	1,415,000
Hemoglobin grs. per 100 cc.	14.29	18.64	11.38	2.6
Hematocrit per cent cells	46.53	57.00	37.50	11.00
Reticulocytes per cent R.B.C.	0.46	2.3	0.0	1.9
Leukocytes per cmm.	13,750	25,250	4,400	12,050
Mean Corpuscular Volume (cubic micra)	69.45	77.2	61.7	11.0
Mean Corpuscular Hemoglobin (gamma gamma)	21.41	24.8	19.0	4.0
Mean Corpuscular Hemoglobin Conc. (per cent)	30.94	35.5	27.5	4.4

observations. These variations are definitely beyond the limits of observational error. In Tables III to IX inclusive are shown in detail the erythrocyte counts, the hemoglobin determinations, the hematocrit determinations, the reticulocyte counts, the mean corpuscular volumes, the mean corpuscular hemoglobins, and the mean corpuscular hemoglobin concentrations for the twelve dogs during the preliminary control period of four weeks preceeding operation. These tables also show the same data for the dogs during the nine weeks succeeding operation and during the four weeks following injections of liver extract. From these tables it is apparent that there were no significant changes in the erythrocyte counts, the hemoglobin determinations, the hematocrit determinations, the reticulocyte counts, the mean corpuscular volumes, the mean corpuscular hemoglobins, and the mean corpuscular hemoglobin concentrations in the animals of either the lycopodium spore injected group or the operated control group. It is also apparent from these tables that there were, likewise, no significant changes as the result of the liver extract injections. In other words all of the variations observed were within the limits of normal(see Tables I and II).

From the data of Tables III to IX, Figures I and II were constructed using the arithmetical means for the two groups of dogs. Figures I and II show that there were no significant differences between the mean values of the erythrocytes, the hemoglobin determinations, the hematocrit

TABLE III

ERYTHROCYTE COUNTS OF SPORE INJECTED AND OPERATED CONTROL DOGS
in millions per cmm.

Day	Spore Injected								Operated Control			
	Males				Females				Males		Females	
	#2	#4	#6	#8	#1	#9	#10	#12	#3	#7	#5	#11
0	6.580	7.470	8.930	5.745	7.150	6.570	6.330	5.830	7.055	6.335	6.600	6.575
7	7.100	6.250	8.330	6.230	5.735	6.550	6.860	6.445	6.800	5.330	7.700	6.430
14	7.815	7.320	8.100	6.470	5.880	6.390	6.475	6.605	6.270	6.100	6.285	7.265
21	6.715	6.640	8.320	5.700	6.240	6.580	5.980	6.020	5.575	6.280	6.335	7.715
22	Operation											
28	6.510	7.340	6.675	5.300	6.190	5.755	7.320	5.760	6.135	5.905	5.945	5.935
35	6.470	6.445	7.145	5.490	6.435	6.355	6.250	6.035	7.170	5.905	6.360	6.970
42	6.555	6.880	6.255	6.170	6.290	6.295	6.665	5.775	6.310	5.755	5.930	6.455
49	6.015	6.440	7.440	5.370	7.330	6.000	6.060	5.600	5.790	6.190	5.565	6.935
56	6.610	6.575	7.610	5.275	6.840	7.130	6.165	5.350	6.385	5.810	5.755	8.010
63	6.285	5.680	7.080	5.415	6.390	7.700	6.985	5.985	6.620	5.605	5.680	6.590
70	6.735	6.685	6.930	4.880	8.470	6.960	6.790	5.970	5.300	5.525	6.315	7.050
77	7.380	6.000	7.570	5.240	7.585	6.465	5.675	5.600	5.490	5.480	5.445	6.930
84	6.705	7.120	6.675	5.445	7.980	6.490	7.110	5.665	5.805	4.870	5.850	6.380
Liver Extract Injected 85th through 89th Days												
88	7.415	5.850	7.325	5.770	6.320	7.285	6.685	6.775	5.455	5.005	5.605	6.770
91	6.725	6.325	6.955	5.355	7.370	7.290	6.430	6.190	6.400	4.945	6.445	6.715
95	6.965	7.365	5.655	5.555	6.985	7.235	6.785	6.005	6.150	5.470	6.410	7.150
98	6.740	6.390	6.780	5.530	7.690	7.545	6.240	6.120	5.605	5.125	6.510	6.670
101	6.740	6.830	7.425	5.490	7.390	7.175	6.975	6.650	6.105	6.620	6.465	6.815
105	6.575	6.015	6.875	5.175	7.895	8.185	6.890	6.680	5.765	5.010	6.780	6.650
108	6.480	6.535	8.020	5.430	7.300	6.775	6.790	6.350	6.095	5.075	7.065	7.300
112	6.965	7.140	7.205	5.230	7.910	5.990	6.980	6.535	6.055	5.410	7.100	6.955

TABLE IV

HEMOGLOBIN VALUES OF SPORE INJECTED AND OPERATED CONTROL DOGS
in grams per 100 cc. of blood (Newcomer scale)

Day	Spore Injected								Operated Control			
	Males				Females				Males		Females	
	#2	#4	#6	#8	#1	#9	#10	#12	#3	#7	#5	#11
0	16.3	15.1	18.6	11.8	13.6	13.0	14.9	14.0	16.9	13.4	14.0	13.4
7	14.8	13.1	16.6	13.5	11.5	14.5	15.0	13.7	16.2	13.2	14.3	14.3
14	15.5	15.7	17.2	12.9	11.4	13.1	15.1	14.0	13.9	13.4	13.3	15.4
21	15.4	15.5	16.5	12.1	12.6	14.0	12.8	13.7	16.2	13.7	13.7	15.0
22	Operation											
28	14.1	15.0	14.7	12.1	13.1	13.3	14.8	12.9	15.1	14.1	11.9	13.7
35	13.3	14.2	13.3	11.7	12.8	12.4	14.4	13.7	15.7	12.6	12.7	14.8
42	13.7	14.1	13.1	12.3	14.1	14.8	14.3	12.0	14.2	12.3	12.1	13.2
49	13.3	13.1	13.9	11.0	13.7	13.0	14.0	12.1	13.7	12.1	12.0	14.2
56	13.8	13.4	13.8	11.7	14.0	14.4	14.5	11.1	14.1	11.5	11.7	15.2
63	12.7	13.4	14.2	9.8	13.9	14.1	15.2	12.1	14.1	10.7	12.0	14.2
70	14.1	14.0	13.6	9.9	15.5	14.0	14.5	13.5	13.7	11.6	11.9	14.7
77	15.2	12.9	15.0	10.2	15.3	14.2	12.1	11.5	12.6	10.1	11.7	14.2
84	13.8	14.1	14.0	11.5	15.8	14.5	14.8	11.6	12.5	9.9	11.9	13.8
Liver Extract Injected 85th through 89th Days												
88	15.4	13.3	14.2	10.7	13.9	15.7	14.1	13.6	12.1	10.2	10.3	13.6
91	15.3	14.3	13.3	10.8	15.1	14.8	14.6	13.3	13.6	9.7	12.2	13.9
95	14.2	13.8	11.6	11.6	15.0	13.5	14.2	12.9	12.5	9.6	11.8	14.3
98	13.2	13.2	14.5	11.6	15.2	11.6	15.2	14.6	10.3	10.6	12.0	13.8
101	14.0	14.0	14.7	11.3	16.0	15.4	14.6	13.8	12.4	9.8	13.0	12.8
105	14.0	13.4	14.1	11.2	17.0	15.0	14.0	13.6	12.1	9.8	12.3	13.3
108	14.7	14.0	15.5	12.0	16.0	14.3	15.1	13.0	12.9	9.6	13.0	14.4
112	13.9	14.1	14.6	11.3	15.3	13.8	15.1	13.3	12.3	9.9	13.3	14.3

TABLE V

HEMATOCRIT DETERMINATIONS OF SPORE INJECTED AND OPERATED CONTROL DOGS
in per cent cells

Day	Spore Injected								Operated Control			
	Males				Females				Males		Females	
	#2	#4	#6	#8	#1	#9	#10	#12	#3	#7	#5	#11
0	49.50	48.25	57.00	41.50	46.25	45.25	49.75	45.00	54.00	43.00	45.50	42.75
7	45.00	43.00	53.50	43.50	37.50	48.75	50.00	46.00	52.50	41.00	48.00	47.00
14	49.75	54.00	55.00	44.25	39.75	45.00	44.00	40.75	45.25	42.00	42.50	48.50
21	46.75	48.00	52.25	42.25	42.50	46.50	41.00	43.75	50.50	43.75	42.00	49.00
22	Operation											
28	45.00	48.25	45.25	36.75	41.75	42.25	49.75	40.50	46.50	41.50	38.25	42.25
35	45.00	45.75	45.75	38.00	43.75	41.25	41.50	44.00	48.00	41.50	41.75	43.25
42	46.75	45.50	46.25	42.00	47.25	49.50	46.75	39.75	45.75	41.00	39.50	42.50
49	44.00	42.50	43.00	38.25	48.00	44.00	46.50	39.00	45.00	39.00	39.25	44.00
56	43.75	43.00	45.00	39.75	46.00	47.75	46.50	37.75	44.25	39.75	39.25	48.75
63	41.25	44.75	46.25	35.00	44.75	47.75	49.25	42.50	44.50	36.25	38.75	44.00
70	46.50	44.75	43.75	34.50	54.00	46.50	48.75	43.75	44.25	38.75	40.50	47.00
77	48.75	42.50	49.75	38.75	51.50	45.25	42.75	39.75	40.50	36.00	39.50	45.75
84	45.25	47.25	46.25	40.00	54.25	47.25	47.00	39.75	41.00	31.50	39.25	42.00
Liver Extract Injected 85th through 89th Days												
88	49.25	43.00	46.25	38.75	46.00	48.50	45.00	45.25	39.50	33.25	34.50	44.00
91	48.25	44.25	44.25	39.00	51.50	47.25	46.50	42.50	42.00	41.00	32.50	43.00
95	46.75	47.00	39.75	40.00	51.00	46.25	47.00	41.25	40.00	33.00	37.75	45.00
98	43.75	45.25	47.75	39.75	54.75	49.00	48.50	42.75	38.00	34.75	41.00	45.00
101	45.50	45.75	47.50	40.00	50.75	51.25	49.50	44.75	41.00	37.00	43.00	42.50
105	44.75	43.50	47.50	40.00	53.25	48.50	46.50	45.50	40.00	34.00	43.00	43.00
108	48.00	46.50	51.50	40.25	50.00	48.00	50.25	44.50	42.25	34.50	41.25	48.75
112	46.00	46.50	48.50	38.00	49.00	41.50	48.50	43.75	41.75	35.50	42.25	47.00

TABLE VI

RETICULOCYTE COUNTS OF SPORE INJECTED AND OPERATED CONTROL DOGS
in per cent

Day	Spore Injected								Operated Control			
	Males				Females				Males		Females	
	#2	#4	#6	#8	#1	#9	#10	#12	#3	#7	#5	#11
0	0.0	0.6	0.2	1.2	1.4	0.0	0.0	0.5	0.2	0.1	0.0	0.2
3	0.1	0.3	0.1	1.8	1.5	0.0	0.4	0.3	0.1	0.8	0.1	0.6
7	0.1	0.1	0.2	1.8	0.4	0.6	0.7	0.5	0.3	0.0	0.0	0.3
10	0.0	1.0	0.0	2.3	1.7	0.2	0.6	0.1	0.2	0.3	0.0	0.4
14	0.7	0.8	0.0	1.2	1.1	0.5	0.4	0.1	0.2	0.4	0.0	0.2
17	0.5	0.3	0.0	0.4	1.6	0.6	0.5	0.1	0.4	0.3	0.2	0.1
21	0.2	0.5	0.0	1.2	0.9	1.2	0.7	0.1	0.4	0.0	0.4	0.2
22	Operation											
24	0.7	0.4	0.2	0.3	0.4	0.3	0.5	0.2	0.1	0.5	0.8	0.2
28	0.3	0.4	0.0	0.0	0.4	0.5	0.6	0.1	0.4	0.4	0.0	0.2
31	0.1	0.3	0.0	0.4	0.4	0.3	0.8	0.0	0.0	0.3	0.8	0.5
35	0.0	0.9	0.0	0.9	1.1	0.1	0.3	0.2	0.2	0.7	1.9	0.4
38	0.2	0.1	0.0	1.2	0.8	0.7	0.3	0.0	0.1	0.4	1.3	0.1
42	0.3	0.3	0.4	0.9	1.1	0.7	0.4	0.2	0.1	0.0	0.7	0.2
45	0.0	0.1	0.0	0.1	0.4	0.4	0.0	0.0	0.3	0.4	0.3	0.0
49	0.4	1.1	0.1	1.1	0.8	0.7	0.1	0.3	0.3	1.2	1.5	0.1
52	0.0	1.2	0.3	0.8	0.6	0.1	0.2	0.1	0.0	0.2	0.2	0.1
56	0.1	1.2	0.5	1.0	1.0	0.4	0.1	0.7	0.3	0.7	1.2	0.2
59	0.2	0.8	0.0	0.6	0.6	0.0	0.1	0.8	0.1	0.7	0.2	0.0
63	0.2	1.0	0.2	0.8	0.8	0.5	0.1	0.1	0.1	1.2	0.3	0.0
66	0.4	0.8	0.3	0.4	1.0	0.0	0.0	0.5	0.0	0.5	0.1	0.1
70	0.3	1.3	0.3	1.2	1.2	0.3	0.2	0.3	0.2	1.3	0.4	0.5
73	0.3	0.4	0.1	1.3	0.4	0.3	0.2	0.5	0.1	0.5	0.5	0.7
77	0.4	1.1	0.1	1.8	1.0	0.4	0.1	0.3	0.5	1.6	0.6	0.8
80	0.4	0.7	0.0	1.2	0.7	0.4	0.0	0.1	0.5	0.6	0.2	0.2
84	0.1	0.8	0.0	0.8	0.8	1.0	0.0	0.0	0.2	1.0	0.2	0.1

TABLE VI (Concluded)

Day	Spore Injected								Operated Control			
	Males				Females				Males		Females	
	#2	#4	#6	#8	#1	#9	#10	#12	#3	#7	#5	#11
Liver Extract Injected 85th through 89th Days												
85	0.3	0.9	0.1	0.6	0.5	0.2	0.0	0.7	0.2	0.8	0.2	0.2
86	0.2	0.8	0.0	0.8	0.7	0.1	0.0	0.4	0.1	0.5	0.4	0.3
87	0.4	0.7	0.2	0.5	0.8	0.3	0.0	0.2	0.2	0.8	0.1	0.1
88	0.2	1.3	0.1	1.2	0.5	0.0	0.1	0.2	0.3	1.5	0.0	0.1
89	0.5	0.8	0.3	0.6	1.0	0.3	0.0	0.7	0.0	1.7	0.1	0.1
90	0.2	1.6	0.4	0.8	0.1	0.0	0.1	0.4	0.2	0.8	0.4	0.2
91	0.5	1.1	0.4	1.2	0.3	0.3	0.2	0.2	0.2	1.5	0.1	0.1
92	0.3	1.4	1.1	1.1	0.3	0.0	0.2	0.2	0.2	0.4	0.0	0.4
93	0.0	1.3	0.5	1.9	0.3	0.1	0.4	0.0	0.2	1.9	0.0	0.1
94	0.3	1.5	0.0	1.2	0.1	0.1	0.0	0.1	0.0	1.4	0.1	0.4
95	0.1	0.8	0.2	0.7	0.4	0.0	0.3	0.5	0.1	0.4	0.6	0.1
96	0.1	1.1	0.0	0.3	0.2	0.1	0.3	0.0	0.2	1.1	0.3	0.1
97	0.5	0.9	0.2	0.7	0.1	0.2	0.0	0.1	0.0	0.2	0.1	0.4
98	0.2	0.2	0.0	1.0	0.5	0.2	0.1	0.1	0.0	2.1	0.4	0.1
99	0.2	1.2	0.1	0.5	0.4	0.1	0.1	0.6	0.2	1.2	0.0	0.6
100	0.0	0.6	0.1	0.4	0.3	0.2	0.0	0.2	0.0	1.5	0.0	0.4
101	0.1	0.7	0.1	0.3	0.1	0.1	0.0	0.4	0.1	1.0	0.2	0.6
102	0.0	1.0	0.1	0.4	0.5	0.2	0.1	0.4	0.2	0.8	0.2	0.3
103	0.0	1.7	0.0	0.6	0.3	0.1	0.1	0.2	0.4	0.6	0.0	0.1
104	0.1	0.5	0.0	0.9	0.1	0.0	0.1	0.2	0.1	0.4	0.1	0.6
105	0.3	0.8	0.0	1.4	0.6	0.4	0.4	0.3	0.2	1.0	0.1	0.3
106	0.0	0.9	0.1	1.2	0.0	0.3	0.2	0.4	0.4	0.7	0.1	0.1
107	0.0	0.3	0.1	0.4	0.6	0.1	0.3	0.4	0.0	1.2	0.2	0.1
108	0.0	1.0	0.1	2.8	0.2	0.4	0.2	0.8	0.1	0.8	0.4	0.0
109	0.0	1.0	0.1	1.5	0.2	0.4	0.3	0.2	0.2	0.2	0.4	0.4
110	0.1	0.7	0.0	1.2	0.1	0.0	0.2	0.4	0.0	0.4	0.3	0.2
111	0.0	1.7	0.3	1.8	0.0	0.3	0.0	0.2	0.2	0.2	0.0	0.1
112	0.2	1.2	0.2	1.4	0.3	0.1	0.1	0.3	0.2	0.5	0.2	0.4

TABLE VII

MEAN CORPUSCULAR VOLUMES OF SPORE INJECTED AND OPERATED CONTROL DOGS
in cubic micra

Day	Spore Injected								Operated Control			
	Males				Females				Males		Females	
	#2	#4	#6	#8	#1	#9	#10	#12	#3	#7	#5	#11
0	73.7	64.6	63.8	72.2	64.7	68.9	72.8	77.2	76.6	67.9	68.9	65.0
7	63.4	68.8	64.2	69.8	65.4	74.4	72.8	69.2	77.2	76.9	68.6	73.1
14	63.7	73.8	67.9	68.4	67.6	70.4	69.5	61.7	72.1	68.9	67.6	66.8
21	69.6	72.3	62.8	74.1	69.6	70.7	68.6	72.7	75.1	69.7	66.3	63.5
22	Operation											
28	69.1	65.7	67.8	69.4	67.5	73.4	68.0	70.3	75.8	70.3	64.3	71.2
35	69.6	71.0	64.0	70.1	68.0	64.9	66.4	72.9	64.2	70.3	65.6	62.1
42	71.2	66.1	73.9	68.1	75.1	78.6	70.1	68.8	72.5	71.0	66.6	65.8
49	73.2	68.0	57.8	71.2	65.5	73.3	76.6	69.6	77.7	63.0	70.5	63.4
56	66.0	65.4	59.1	75.7	67.2	67.0	75.4	70.6	69.3	68.4	68.2	60.9
63	65.6	78.8	65.3	64.6	70.0	62.0	70.5	71.0	67.2	64.7	68.2	66.8
70	69.2	66.9	63.1	70.7	65.8	66.8	71.8	73.3	70.2	70.1	63.4	66.7
77	64.7	70.8	65.7	73.5	67.9	65.4	75.3	71.0	72.0	65.7	72.5	66.0
84	67.5	65.0	69.3	73.5	67.9	72.8	66.1	70.3	70.6	64.7	67.1	65.8
Liver Extract Injected 85th through 89th Days												
88	66.4	73.5	63.3	71.0	72.8	66.7	67.3	66.8	72.4	66.5	61.7	65.0
91	71.8	70.0	63.6	70.5	69.9	64.8	72.2	68.7	65.6	65.7	63.6	64.0
95	67.2	63.8	70.3	72.0	73.0	63.9	69.4	68.7	64.7	60.3	58.7	64.3
98	64.9	65.3	70.0	71.9	71.1	64.9	77.7	69.8	67.8	67.8	61.6	67.5
101	67.4	67.0	65.6	72.9	62.7	72.3	71.0	67.3	65.8	65.9	66.5	62.4
105	68.2	72.3	69.0	72.3	67.6	59.2	67.5	68.1	69.4	67.9	63.4	64.7
108	74.1	71.2	64.2	72.3	68.5	70.9	74.0	70.1	69.3	68.0	58.4	66.8
112	66.0	65.1	67.3	72.7	61.9	69.3	69.5	66.2	69.0	65.6	59.5	67.6

TABLE VIII

MEAN CORPUSCULAR HEMOGLOBINS OF SPORE INJECTED AND OPERATED CONTROL DOGS
in gamma gamma

Day	Spore Injected								Operated Control			
	Males				Females				Males		Females	
	#2	#4	#6	#8	#1	#9	#10	#12	#3	#7	#5	#11
0	24.8	20.2	20.9	19.8	19.0	19.9	21.8	24.0	24.0	21.1	21.2	20.4
7	20.8	21.0	19.9	21.6	20.0	22.1	21.8	20.6	23.8	24.8	20.4	22.4
14	19.7	21.3	21.2	19.9	19.3	20.5	23.3	21.1	22.2	21.9	21.2	21.2
21	21.7	23.3	19.8	21.2	20.2	21.3	21.0	22.8	24.6	21.8	21.6	19.4
22	Operation											
28	21.7	20.4	22.0	22.9	21.2	21.1	20.2	22.5	24.8	23.7	20.1	23.1
35	20.6	22.1	18.6	21.3	19.8	19.5	23.0	22.7	21.9	21.3	20.0	21.3
42	20.9	20.5	21.0	19.9	21.4	23.5	21.5	20.8	20.9	21.4	20.5	20.4
49	22.2	20.4	18.6	20.5	18.7	21.6	23.1	21.6	23.6	19.5	21.6	20.5
56	20.9	20.4	18.1	22.2	20.5	20.2	23.6	20.7	22.1	19.9	20.4	19.0
63	20.2	23.6	20.1	18.0	21.7	18.3	21.8	20.2	21.3	19.0	21.1	21.4
70	21.0	20.9	19.1	20.3	18.3	20.1	21.3	22.6	21.7	21.0	18.9	20.8
77	20.6	21.6	21.0	19.3	20.2	20.4	21.3	20.6	23.0	18.5	21.5	20.6
84	20.6	19.7	19.8	21.0	19.8	22.4	20.8	20.5	21.5	20.3	20.3	21.6
Liver Extract Injected 85th through 89th Days												
88	20.7	22.8	19.4	19.5	22.0	21.5	21.0	20.0	22.2	20.3	18.4	20.0
91	22.8	22.5	19.1	19.5	20.5	19.9	22.7	21.4	21.3	19.7	19.0	20.7
95	20.3	18.7	20.5	20.9	21.5	18.7	20.9	21.5	20.2	17.6	18.4	20.0
98	19.6	19.0	21.4	20.9	19.7	19.3	25.0	20.9	18.4	20.7	18.5	20.7
101	20.6	20.5	20.4	20.6	20.1	21.7	20.9	20.7	20.3	19.6	19.0	19.5
105	21.2	22.3	20.4	21.7	21.4	18.3	20.3	20.4	21.1	19.5	19.1	19.3
108	22.6	21.4	19.3	22.1	21.9	21.3	22.2	20.4	21.1	18.9	18.4	19.7
112	20.0	19.7	20.3	21.7	19.3	22.4	21.7	20.4	20.3	18.3	18.7	20.6

TABLE IX

MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATIONS OF SPORE INJECTED AND OPERATED CONTROL DOGS
in per cent

Day	Spore Injected								Operated Control			
	Males				Females				Males		Females	
	#2	#4	#6	#8	#1	#9	#10	#12	#3	#7	#5	#11
0	33.0	31.3	33.1	27.5	29.4	28.8	29.8	31.1	31.3	31.1	30.7	31.4
7	32.8	30.5	31.0	30.9	30.6	29.7	29.9	29.8	30.9	29.8	32.2	30.5
14	31.3	29.1	31.3	29.9	28.6	29.2	34.3	34.2	30.8	31.8	31.4	31.8
21	31.1	32.3	31.5	28.6	29.7	30.1	31.2	31.3	32.1	31.2	32.5	30.6
22	Operation											
28	31.3	31.0	32.4	33.0	31.7	31.5	29.8	32.0	32.4	34.0	32.5	32.5
35	29.6	31.1	29.1	30.4	29.1	30.0	34.7	31.1	34.2	30.3	30.5	34.3
42	29.3	31.0	28.4	29.3	29.8	29.9	30.7	30.2	31.0	30.1	30.7	31.0
49	30.3	30.9	32.2	28.8	28.5	29.4	30.1	31.0	30.4	31.0	30.6	32.3
56	31.6	31.2	30.5	29.4	30.5	30.2	31.2	29.3	31.8	29.0	29.9	31.2
63	30.7	30.0	30.7	27.9	31.0	29.5	30.9	28.5	31.6	29.4	30.9	31.8
70	30.4	31.3	31.0	28.8	28.6	30.1	29.7	30.9	30.9	30.0	29.5	31.3
77	31.1	30.4	30.3	26.2	29.6	31.2	28.3	29.0	31.1	28.2	29.7	31.1
84	30.4	29.7	30.3	28.7	29.1	31.0	31.4	28.9	30.4	31.4	30.3	32.8
Liver Extract Injected 85th through 89th Days												
88	31.2	31.0	30.7	27.6	30.2	32.3	31.2	30.0	30.6	30.6	29.8	30.8
91	31.8	32.3	30.1	28.4	29.4	30.6	31.4	31.2	32.4	29.9	29.8	32.3
95	30.3	29.4	28.9	28.9	29.4	29.1	30.1	31.3	31.1	29.2	31.8	31.7
98	30.2	29.1	30.4	29.1	27.7	29.8	32.4	30.0	27.1	30.5	29.3	30.7
101	30.7	30.6	31.0	28.2	31.4	30.0	29.5	30.7	30.2	29.5	28.6	31.3
105	31.2	30.9	29.6	28.1	31.8	30.9	30.1	29.9	30.3	28.7	30.2	29.9
108	30.8	30.0	30.0	29.9	31.9	29.8	30.1	29.1	30.5	27.8	31.5	29.5
112	30.2	30.2	30.2	29.6	30.8	32.3	31.2	30.8	29.4	27.8	31.4	30.4

FIGURE I

AVERAGE ERYTHROCYTE COUNTS, HEMOGLOBIN VALUES, HEMATOCRIT DETERMINATIONS, AND RETICULOCYTE COUNTS OF EIGHT SPORE INJECTED AND FOUR OPERATED CONTROL DOGS

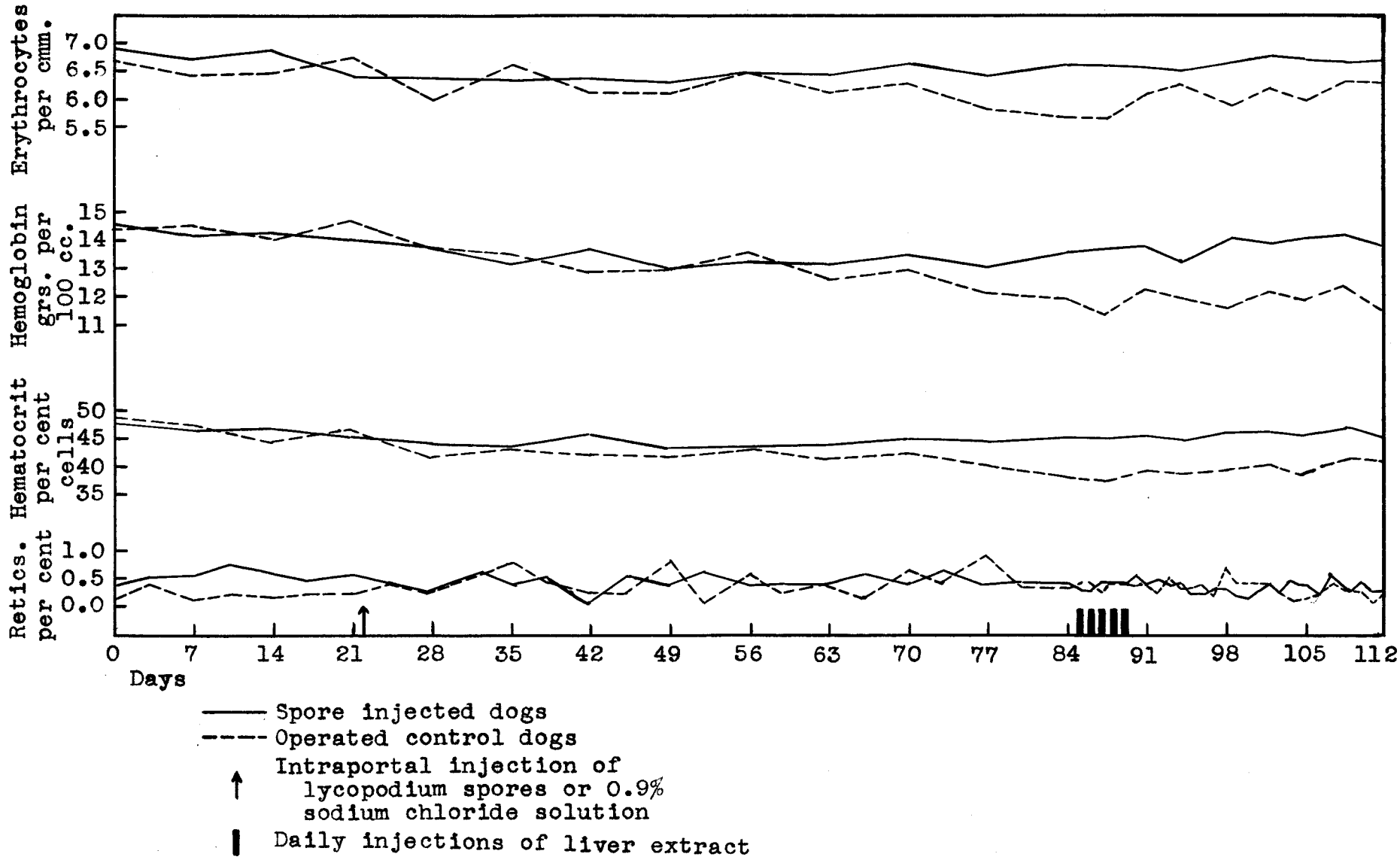
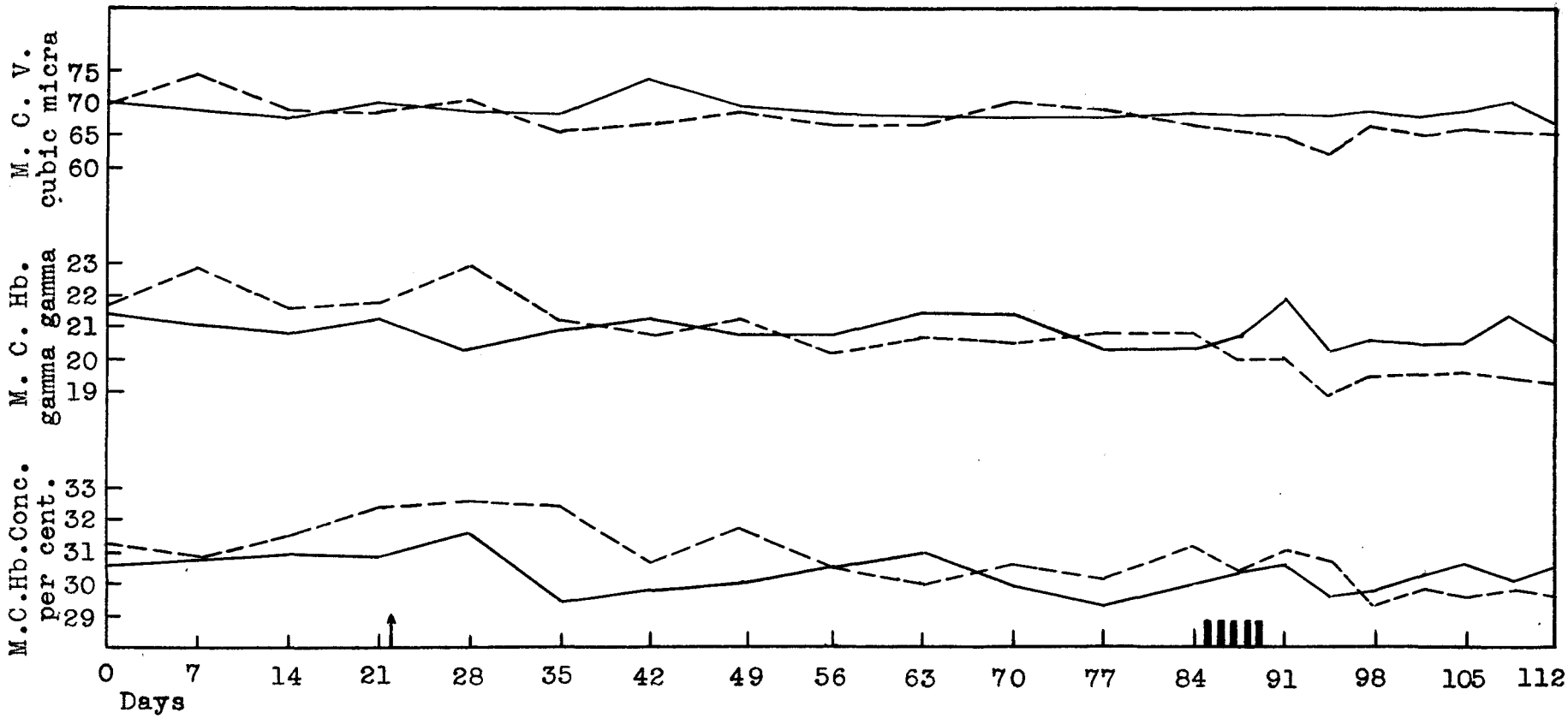


FIGURE II

AVERAGE MEAN CORPUSCULAR VOLUMES, MEAN CORPUSCULAR HEMOGLOBINS, AND MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATIONS OF EIGHT SPORE INJECTED AND FOUR OPERATED CONTROL DOGS



Legend as in Figure I

determinations, the reticulocyte counts, the mean corpuscular volumes, the mean corpuscular hemoglobins, and the mean corpuscular hemoglobin concentrations of the operated control and spore injected groups of dogs during the four weeks preliminary control period. Similar curves (not included in this thesis) for the individual animals also show that there were no significant differences between data of the two groups of dogs. Figures I and II demonstrate further that during the nine weeks subsequent to operation there were no significant differences between the mean values of the data for the spore injected and operated control groups of animals. After the injection of liver extract there were likewise no significant differences between the mean values of the two groups of animals.

Table X, consisting of the average erythrocyte counts and average mean corpuscular volumes of the twelve dogs during the preliminary control period, indicates a certain amount of correlation between the erythrocyte count and the mean corpuscular volume for most of the dogs. In particular, dogs #2, #6, and #11 have the highest average erythrocyte counts and the lowest average mean corpuscular volumes. On the other hand, dogs #1, and #3 fail to show evidence of such a correlation. These findings are evident also from Figure III.

Blood films made on the day of the last count for each dog were negative for Bartonella canis(39,40).

In Table XI are shown the leukocyte counts for the

TABLE X

AVERAGE ERYTHROCYTE COUNTS AND MEAN CORPUSCULAR VOLUMES OF THE SPORE INJECTED AND OPERATED CONTROL DOGS DURING THE FOUR WEEKS PREOPERATIVE CONTROL PERIOD

Dog	Erythrocytes millions per cmm.	Mean Corpuscular Volume cubic micra
#6	8.4	64.7
#2	7.1	67.3
#11	7.0	67.1
#4	6.9	69.9
#3	6.7	75.3
#5	6.6	67.9
#10	6.5	70.9
#9	6.5	71.1
#12	6.2	70.2
#1	6.2	66.8
#8	6.0	71.1
#7	6.0	70.8

FIGURE III

RELATION OF AVERAGE ERYTHROCYTE COUNTS TO MEAN CORPUSCILAR VOLUMES DURING THE PREOPERATIVE CONTROL PERIOD

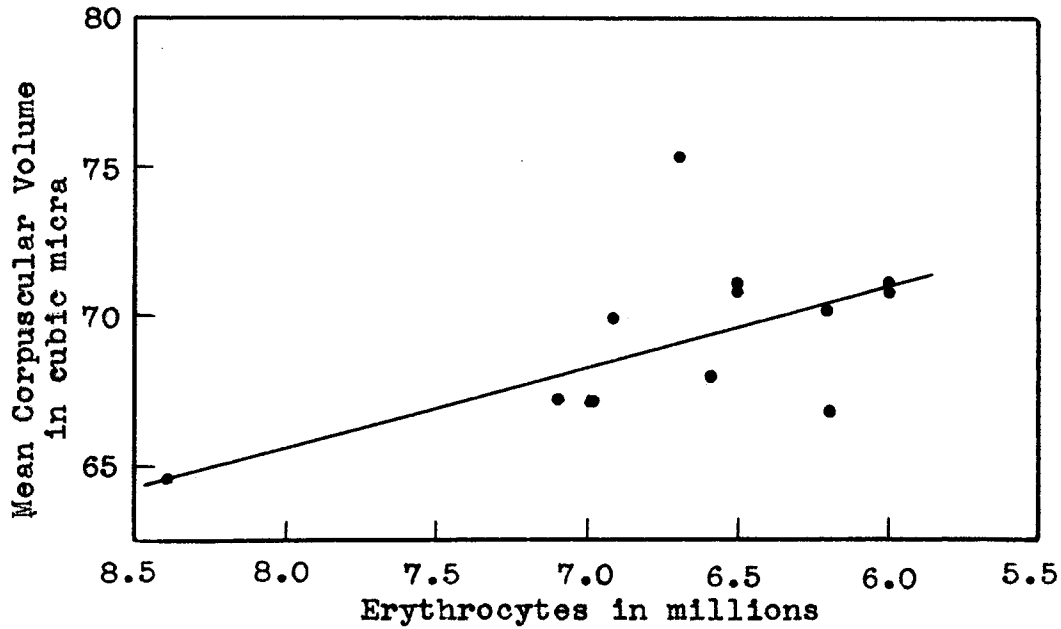


TABLE XI

LEUKOCYTE COUNTS OF SPORE INJECTED AND OPERATED CONTROL DOGS
in thousands per cmm.

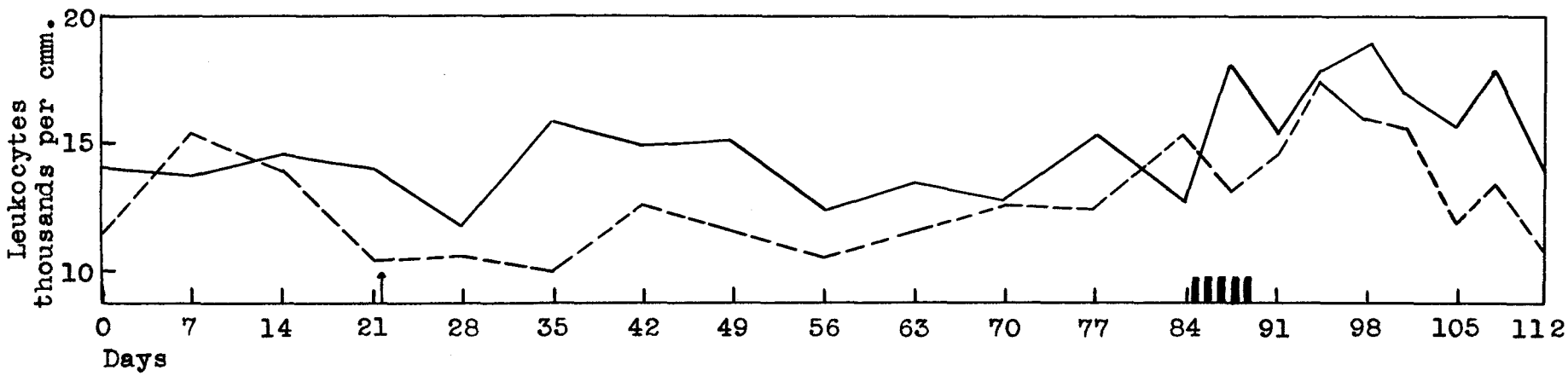
Day	Spore Injected								Operated Control			
	Males				Females				Males		Females	
	#2	#4	#6	#8	#1	#9	#10	#12	#3	#7	#5	#11
0	13.35	18.45	4.60	8.75	18.50	17.30	11.70	19.95	9.55	15.45	4.50	16.75
7	14.55	17.50	8.65	18.30	11.80	8.85	12.50	17.60	13.35	24.90	9.95	13.40
14	12.80	15.35	10.95	25.25	23.85	12.40	4.40	13.75	11.75	17.75	12.50	14.75
21	11.00	15.10	15.50	23.75	9.40	10.50	8.30	18.90	11.00	12.75	8.65	9.55
22	Operation											
28	8.30	14.10	8.60	13.55	17.10	10.15	10.50	11.15	8.50	17.50	7.10	9.40
35	9.00	11.90	4.80	27.30	25.65	18.10	10.10	19.65	6.80	14.40	10.90	7.35
42	10.60	12.90	11.85	30.05	19.95	15.20	5.75	12.50	14.40	7.75	12.80	15.55
49	15.20	11.10	10.40	19.70	17.70	21.60	10.70	15.20	9.05	11.90	12.15	12.10
56	8.85	12.40	10.25	17.60	15.35	13.95	8.05	15.00	6.30	11.55	12.35	10.25
63	12.20	10.20	7.30	28.85	13.85	17.10	9.40	17.30	7.75	13.15	11.95	13.85
70	12.35	6.35	12.75	22.60	13.35	12.95	9.65	12.50	10.00	14.35	11.40	14.85
77	15.15	14.40	19.55	24.50	11.55	8.60	7.65	15.20	11.95	14.90	10.75	12.20
84	14.40	13.80	8.15	20.90	17.30	7.25	10.55	9.35	10.85	21.10	12.90	16.60
Liver Extract Injected 85th through 89th Days												
88	19.55	18.25	22.40	23.30	14.50	17.20	9.80	19.90	14.00	8.40	18.20	12.20
91	13.20	18.50	10.65	21.75	16.15	16.25	8.25	18.90	13.70	13.45	15.30	15.90
95	20.90	11.85	9.35	38.30	22.00	12.90	11.85	13.95	13.15	14.80	18.25	23.90
98	17.50	15.95	11.00	33.65	26.05	17.35	12.80	17.35	16.00	16.65	15.85	15.40
101	14.20	17.95	6.50	39.65	17.50	9.85	15.70	13.90	18.25	13.90	17.55	12.85
105	16.60	12.20	12.40	29.10	15.00	9.95	18.90	9.55	11.10	12.20	10.65	13.70
108	13.50	13.70	17.70	44.85	13.30	11.10	16.80	11.15	10.35	18.15	10.40	14.95
112	6.45	8.55	9.40	39.00	9.95	10.20	8.20	18.25	12.70	11.60	6.15	12.30

twelve dogs during the four weeks preliminary control period. It is apparent that these data, summarized in Table II, fall within the limits of normal indicated in Table I. Moreover, there were no significant changes in the leukocyte counts of any of the dogs during the nine weeks following operation or during the four weeks following the injections of liver extract. Throughout the experiment, dog #8, an apparently healthy animal, maintained a leukocyte count considerably higher than the other animals. The reason for this finding is unknown. Figure IV was constructed from the data of Table XI using the arithmetical means of the data of the operated control group and spore injected group. The curves show that there were no significant differences between the averaged leukocyte counts of the two groups of animals during the four weeks preliminary control period; similarly, there were no significant differences between the averaged counts of the two groups during the nine weeks following operation or during the four weeks succeeding liver extract injections. The somewhat higher level of the spore injected group is due to the high counts of dog #8.

The greyish-red layer of cells capping the cellular column of the hematocrit tubes after centrifuging has been mentioned before. This layer of cells, when smeared on a slide and stained, was found to be composed of erythrocytes as well as leukocytes. Since the proportion of red and white cells was not found to be constant, an effort to estimate accurately the

FIGURE IV

AVERAGE LEUKOCYTE COUNTS OF EIGHT SPORE INJECTED
AND FOUR OPERATED CONTROL DOGS



Legend as in Figure I

number of leukocytes per volume of blood by the size of the cap was unsuccessful.

Liver function as determined by the Rose Bengal dye excretion test is expressed in per cent according to the colorimetric formula proposed by Delprat and Stowe(37), which is so constructed that 100% is normal for the normal human liver. From Table XII it is seen that during the four weeks preliminary control period values between 77% and 137% were obtained. The average of the data for the twelve dogs during this period was 107%. Taking these values to represent normal variations, it is evident that there were no significant variations from the above limits during either the nine weeks succeeding operation or the four weeks following the injections of liver extract. From Figure V, constructed from the arithmetical means of the data for the operated control and spore injected groups of dogs, it is evident that there were no significant differences between the control and spore injected groups of animals during the four weeks preliminary control period. Further, there were no significant differences between the two groups of animals during the nine weeks following operation or the four weeks subsequent to the injections of liver extract.

The blood chemistry studies showed no significant changes in non-protein nitrogen, urea, blood sugar, or CO_2 combining power of the plasma in any animal during the entire course of the experiment. The qualitative van den Bergh test

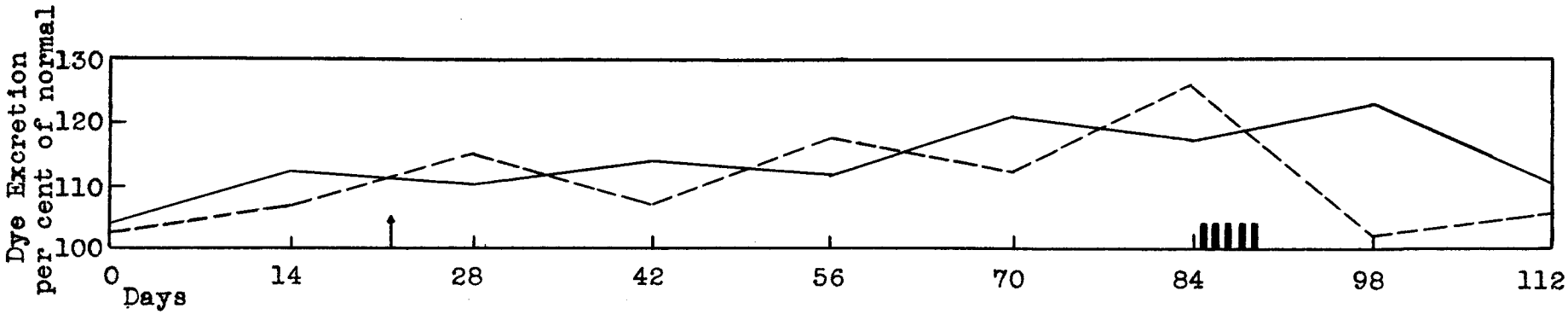
TABLE XII

ROSE BENGAL TEST RESULTS OF SPORE INJECTED AND OPERATED CONTROL DOGS
in per cent

Day	Spore Injected								Operated Control			
	Males				Females				Males		Females	
	#2	#4	#6	#8	#1	#9	#10	#12	#3	#7	#5	#11
0	91	102	120	125	99	108	96	96	110	129	96	77
14	106	106	111	137	130	129	94	99	101	112	105	110
22	Operation											
28	120	120	91	116	134	85	122	126	117	113	124	107
42	116	101	89	126	123	109	117	102	90	112	115	111
56	112	121	118	139	112	115	104	133	121	123	97	128
70	129	141	133	130	115	135	90	112	128	101	97	119
84				92	109	133			144	132	112	114
Liver Extract Injected 85th through 89th Days												
98	117	134	116	127	122	113	113	136	116	76	91	125
112	117	99	124	96	93	123	105	124	124	86	101	119

FIGURE V

AVERAGE ROSE BENGAL LIVER FUNCTION TESTS FOR
EIGHT SPORE INJECTED AND FOUR
OPERATED CONTROL DOGS



Legend as in Figure I

throughout the experiment was recorded as negative on all dogs. Discoloration of the sclerae was never observed in any animals.

Throughout the experiment, the dogs on the whole remained in a state of good health. The abdominal operative wounds closed by primary healing with two exceptions, dogs #5, and #11(both of the operated control group); these, however, healed upon incision and open drainage. Dog #10(spore injected group) developed a keratitis on the eighty-eighth day which subsided with local treatment on the ninety-second day. This animal subsequently went into estrus on the ninety-fifth day. Dog #1(spore injected group) went into estrus on the one hundred sixth day. During the 112 days of the experiment the spore injected and control animals lost an average of 8.1% and 8.8% respectively of their initial weight.

As previously noted, all the animals except three of the spore injected group were sacrificed and autopsied. The gross findings were entirely unremarkable except for a slight gritty sensation obtained from the knife edge applied to the cut surface of the spore injected livers. The weight of the livers of the spore injected animals was found comparable to the weight of the livers of the control animals: an average of 42 grams per kg. to 40 grams per kg. respectively. There were observed no varices, ulcers or other pathological changes of the gastro-intestinal tract; ascites was not found. The livers of the spore injected animals presented a normal

gross appearance. The hamstring muscles into which the liver extract was injected showed no evidence of inflammation (foreign protein reaction), degeneration, or scarring.

Sections of the spore injected livers showed that the lobules as a whole were somewhat smaller and stained more lightly than normal at the center and at the periphery. The normal picture for these dogs was obtained from sections of biopsy material removed at operation. The cells at the center of the lobule which stained lightly were smaller, coarsely granular and contained pycnotic or karyolytic nuclei. The cellular outlines were indistinct and the sinusoids appeared wider due to the shrinking of the liver cords. The cells toward the periphery of the lobule appeared normal. Throughout the section the spores were found imbedded in the portal vessels in a fibrous tissue coat containing hyaline material. These vessels were occluded, but there had developed a collateral circulation composed of one or more thin endothelial tubes which penetrated or skirted the occluding mass. The connective tissue of the periportal spaces was increased, but not to the extent of exerting pressure on the newly formed collaterals or the bile ducts.

DISCUSSION:

Analysis of the tabulated results discloses variations of the erythrocyte counts together with more or less parallel variations of the hemoglobins and cell concentrations too extensive to be attributed to error due to the methods employed.

As previously noted, the accuracy of the technique has been verified in man by Wintrobe(34,35,41). In order to exceed errors common to the methods employed, the variations need be of the order of only 2.5%.

The measurements of the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were derived by the same formulae which are applied to the human erythrocyte. These values have been proven accurate and useful(34,35,42). They provide the means by which a macrocytic hyperchromic anemia may be positively identified.

In Table XIII are presented the average erythrocyte, hemoglobin, cell concentration, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration values of normal dogs' blood found in the reports of various investigators together with similar data from Tables I and II. The reasons for the disagreement between the values listed are not entirely clear. As Wintrobe and his associates (41,42,47) and others(49,50) have pointed out, differences in the method of hemoglobin determination, the type of hematocrit used, and the anticoagulant employed account for some of the lack of closer agreement. A similar situation exists relative to the average blood values of normal men(41). In my own work, other factors such as the diurnal variation in number(51,52) and in size(53) of the erythrocyte, and in the hemoglobin content of the erythro-

TABLE XIII

SUMMARY OF AVERAGE ERYTHROCYTE VALUES FOR NORMAL DOGS REPORTED BY DIFFERENT AUTHORS

Author	Erythrocytes (millions per cmm.)	Hemoglobin (grams per 100 cc.)	Hematocrit (per cent cells)	Mean Corpuscular Volume (cubic micra)	Mean Corpuscular Hemoglobin (gamma gamma)	Mean Corpuscular Hemoglobin Conc. (per cent)
Mayerson(43)	6.16	13.0	38.6	59.3	20.0	34.3
Emmons(44)	6.25	16.5*	43.0	69.0	26.4*	38.4*
Ashley and Guest(45)	6.87	16.0	45.6	66.6	23.6	34.9
Powers, Bowie and Howard(46)	7.00	16.0*	45.2	64.2	22.8*	35.3*
Wintrobe, Shumacker, and Schmidt(47)	7.02	14.6	47.3	67.6	21.2	31.4
Leichsenring, et, al.(48)	7.17	14.1	47.7*	66.4*	19.7*	29.6*
Bruner (Table I)	6.53	13.9	45.5	69.7	21.4	30.8
Bruner (Table II)	6.65	14.3	46.5	69.5	21.4	30.9

* Calculated from data

cytes(52,54) have been eliminated as far as possible by the standardized procedure. Various other laboratory animals are subject to similar fluctuations and variations of erythrocytes. In Table XIV summarized from Scarborough's compilation(55) are presented briefly these normal variations.

Leischenring and Honig(56), and Bodansky and Dressler (57) have noted that dogs with high erythrocyte counts tend to have small mean corpuscular volumes. Thus Leischenring and Honig observed a decreased mean corpuscular volume accompanied by a slight polyglobulism following recovery from acute experimental hemorrhage. Bodansky and Dressler state that in their experience, dogs with unusually high erythrocyte counts frequently have small corpuscles. The data obtained from the twelve dogs during the preliminary control period in part confirm these findings in that they show a certain amount of correlation between the number of erythrocytes and the mean corpuscular volume(Table X, Figure III).

The lack of effect of the injection of lycopodium spores on the erythrocytes and hemoglobin shows that this method of liver damage did not produce a quantitative reduction of liver tissue sufficient to cause a deficiency of the anti-pernicious anemia principle, nor did it interfere significantly in a differential manner with the normal role of the liver in hematopoiesis.

Shumacker and Wintrobe(58), found that two of seven

TABLE XIV

VARIATIONS IN THE ERYTHROCYTE COUNTS

OF NORMAL LABORATORY ANIMALS*

	Average (millions per cmm.)	Maximum (millions per cmm.)	Minimum (millions per cmm.)	Greatest range of variation found by one author (millions per cmm.)
Rabbit	5.62	10.0	2.8	5.5
Guinea pig	5.75	11.8	2.5	3.6
Rat	8.50	9.9	6.4	2.6
Mouse	9.70	12.4	7.7	3.5
Dog	7.20	11.8	3.9	5.0
Cat	8.43	14.6	4.8	7.8
Horse	7.80	11.5	5.4	5.5
Pig	6.74	10.1	3.3	6.3
Cattle:Cow	6.62	8.3	4.5	3.1
Ox	6.82	8.6	3.7	2.9
Monkey	5.59	7.1	3.6	3.5
Sheep	10.38	13.0	9.0	2.4
Goat	14.42	17.0	12.2	3.8
Chicken	3.44	5.8	2.0	2.2
Frog	0.46	0.54	0.40	0.14

* Summarized from Scarborough's compilation (55)

dogs, which were subjected to total gastrectomy and severe cirrhosis of the liver produced by carbon tetrachloride, developed a macrocytic hyperchromic anemia shortly before death. This in part suggests that only a small amount of liver tissue is required to maintain a normal blood picture even when the source of the intrinsic factor is reduced.

The total amount of the particular liver extract injected into the animals significantly exceeds the amount necessary to induce remission in the average case of pernicious anemia. The liver extract used was a fresh refined concentrate, 3 cc. derived from 100 grams of fresh liver. Placed on a per kilogram basis, a 60 kg. pernicious anemia patient in relapse would require less than 0.2 cc. per kg. to induce remission. The dogs employed in the present experiment received a total of 1.0 cc. per kg. over a five day period.

As has been found in this experiment, attempts to accelerate erythropoiesis in the normal experimental animal by whole liver or liver extracts have, in general, been unsuccessful. Vaughan and Muller(59) found no effect on the total erythrocytes or the hemoglobin by the addition of whole liver or liver extract to the diet of healthy rats. Landsberg and Thompson(60) failed to find an increased weight or erythrocyte count in guinea pigs after the injection of an active liver extract; Jacobson(61), however, has observed

slight increases in erythrocytes. Adlersburg and Gottsegen (62) have reported a rather unusual finding: working with dogs and rabbits on an ordinary diet they were able to produce a temporary anemia by feeding large doses of German commercial liver preparations effective in pernicious anemia (Hepatrat and Hapatopson).

In the normal human organism, the addition of liver to the diet or the parenteral introduction of liver extracts has been reported as producing slight, if any, effects upon the number of circulating erythrocytes. Cornell(63) has reported no change in the blood of four normal people taking 240 grams of raw liver daily for four weeks. Neidhardt and Bannasch(64), feeding raw liver to eight persons for periods varying between seven and thirty days, found only very slight increases in erythrocytes and hemoglobin in seven of eight instances. Jungmann(65) reported practically no changes with the use of a similar diet. Crane, Howard and Murphy(66) have observed slight increases of erythrocytes and hemoglobin upon the ingestion of the powdered extract of 800 grams of liver daily for fourteen days. On the other hand, Watkins, Johnson and Berglund(67) observed prompt increases in erythrocytes amounting to between eight to twenty per cent in the blood of six normal persons during the ingestion of the equivalent of three kg. of liver over a ten day period; every subject developed symptoms and signs of polycythemia

in varying degrees.

The negative results relative to the erythrocyte counts and hemoglobin values obtained from the injection of liver extract into the eight spore injected dogs and the four operated control dogs agree, therefore, with the majority of reports on the effect of liver extract on the normal members of different species.

The absence of any significant change in the mean corpuscular volumes and mean corpuscular hemoglobins of the spore injected dogs during the nine weeks following operation, indicates again that the sequence of normal hematopoiesis was not interrupted by the experimental procedure. The effect of the injection of liver extract into normal animals studied from the standpoint of the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration has not been reported thus far. It is interesting to note that the liver extract had no effect on the normal sized erythrocytes of either the spore injected or operated control groups. This finding is in agreement with the opinion that the characteristic effect of liver extract on the Price-Jones curve in pernicious anemia(42,68,69) is produced by replacement of the macrocytes with normal sized erythrocytes instead of any direct effect upon the circulating macrocytic erythrocytes.

The average number of reticulocytes, 0.4%, obtained during the preliminary control period compares with values reported by other investigators. Mayerson(43), however,

found an average reticulocyte count of 1.85% in thirty-five dogs. He reported an average of 4% on one dog and found that nine of the thirty-five had average reticulocyte counts higher than the maximal count(2.8%) recorded in my own work. Krumbhaar(70) found 0.6% to be the average in the normal dog.

Jacobson(61,71) has shown that some hematologically normal guinea pigs respond to the administration of whole liver or liver extract by a slight increase in the number of reticulocytes in the circulating blood. The livers of the "reactive" guinea pigs were found to show a reduction in the amount of the stored anti-pernicious anemia principle as established by assay on other "reactive" guinea pigs. It was possible that some or all of the spore injected animals although presenting a normal blood formula, might be in an analagous "reactive" state. The results, however, indicate that there was no significant diminution in the amount of stored anti-pernicious anemia principle in the livers of the spore injected dogs.

In the normal human Neidhardt and Bannasch(64), feeding raw liver, observed that the reticulocyte count rose to a maximum of but 1.2 to 1.7 per cent in seven of eight instances. Crane, Howard, and Murphy(66) have likewise observed negligible changes in reticulocytes upon the ingestion of a powdered extract of liver. The reticulocyte counts of the spore injected dogs following the injections of liver extract agree with the counts of the operated control dogs,

and both are comparable with the results obtained in normal humans, indicating further that the erythropoietic systems of the spore injected dogs were not significantly affected by the experimental procedure.

The average leukocyte count of these dogs during the preliminary control period, 13,750 per c. mm., agrees with counts from other sources. Thus, Scarborough(55) found an average of 11,840, a figure compiled from 788 counts by 67 different investigators. He has tabulated in his report average values on normal dogs ranging between 5,700 and 38,000 per c. mm. Mayerson(43) reported 11,165 per c. mm. as the average of 190 counts on 57 dogs with extremes of 5,560 and 19,920. In man, Garrey and his associates(72,73) have observed increases in the leukocyte count following emotion, pain, movement, exercise and active or passive changes in posture. These factors were reduced to a minimum by the standardized procedure of the experiment. The diurnal variation of the leukocytes was controlled by drawing all blood at approximately the same hour of the morning. As already stated, there is no evident explanation for the high counts of dog #8 during the experiment.

In the case of the dogs whose livers had been injected with spores, there were no indications of the leukocytosis which usually occurs during the process of phagocytosis and removal of autolysing tissue. The unchanged

leukocyte counts of the spore injected dogs during the period following operation is additional evidence of the absence of a blood picture resembling pernicious anemia. However, other pathological states accompanied by a macrocytic hyperchromic anemia do not necessarily exhibit a leukopoenia.

Similarly to the lack of significant response in the leukocyte counts to liver administration noted in this experiment, Neidhardt and Bannasch(64) observed no distinct effect on the leukocytes of normal men on a diet of raw liver. Watkins, Johnson, and Berglund(67), feeding a liver preparation derived from three kg. of fresh liver during a period of ten days, likewise observed no effect on the leukocytes of six normal men. On the other hand, two groups of investigators (74,75) have reported elevated leukocyte counts within seven hours after a single intramuscular injection of liver extract in man, the count returning to normal on the third day. Their data were obtained from leukocyte counts made at one hour intervals and hence are not suitable for comparison with the leukocyte counts of the spore injected and operated control dogs after the liver extract injections.

It was not surprising that the present study of liver function by means of dye excretion produced negative results. Mann and Bollman(76) investigated the liver function in dogs subjected to surgical removal of 70% to 90% of the liver tissue. They employed tests of liver function depending on

the secretion of bile, the relation of the liver to carbohydrate and to protein metabolism, the excretion of dyes, and the detoxifying ability of the liver. With the exception of uric acid metabolism and tests of dye excretion, tests based on these functions produced unreliable results due either to the activity of other tissues or to the ability of the residual liver tissue, which is necessary for life, to maintain these functions within normal limits. In the case of the dyes, phenoltetrachlorphthalein, bromsulphalein and rose bengal, it was necessary to use relatively large amounts in order to demonstrate definite retention in the blood of dogs whose liver tissue had been reduced to the least amount sustaining life. Of the three dyes used, it appears that rose bengal was the most sensitive to hepatic insufficiency.

Using dogs whose livers had been damaged acutely by chemicals, Bollman and Mann(22) have reported that for the most part there was a satisfactory correlation between the degree of injury to the liver, as estimated by the gross appearance and histologic section, and the degree of retention of dye(bromsulphalein). However, some of the dogs with extensive cirrhotic changes gave no evidence of dye retention; conversely, a few normal animals with no demonstrable hepatic lesions showed marked dye retention. They also observed that different activities of the liver may be affected to different degrees. Thus, the destruction of uric

acid is more impaired, than is the formation of urea, by the same degree of liver damage. Here, therefore, is a basis for the possibility that the hematopoietic function of the liver might have been differentially depressed by the procedure employed in this experiment.

Green and Conner(77) investigated liver function in twelve cases of pernicious anemia by means of the phenol-tetrachlorphthalein test, the fructose tolerance test, the serum bilirubin and van den Bergh reaction, and the nitrogen partition of the blood(non-protein nitrogen, urea, uric acid, creatinine, and amino-acid nitrogen). Their positive findings consisted of one instance of a positive direct van den Bergh and serum bilirubin above 5 mgm. per cent following a hemolytic crisis, eight instances of serum bilirubin above the limits of normal, and three instances of moderate dye retention. Thus, liver function as tested clinically is not particularly depressed in cases of pernicious anemia in relapse.

It is probable, therefore, that had a macrocytic hyperchromic anemia developed in the spore injected dogs, liver function measured by the Rose Bengal test would not have been depressed.

Mann(78) has found that in the absence of 70% to 90% of the liver in dogs there develops a characteristic set of blood chemistry findings consisting of hypoglycemia, a loss

of formation of urea together with an increase of amino-acid nitrogen, and an increase in uric acid. The absence of such findings in the spore injected animals suggests that at least 30% of the liver parenchyma was functioning efficiently. It appears that the damage produced by the lycopodium spores was not sufficient to modify the normal role of the liver in carbohydrate and protein metabolism.

Studies of the blood chemistry of pernicious anemia (77,79,80,81) have in general revealed normal or high normal values except for an increased serum bilirubin of 1.4 to 5 mgm. per cent and a "delayed" qualitative van den Bergh test. As already noted above, the spore injected animals continued to show a negative van den Bergh, as did the operated controls. It must be kept in mind, however, that some types of macrocytic hyperchromic anemia are not accompanied by an increased serum bilirubin.

The autopsy findings of the spore injected animals confirmed the evidence pointing to the lack of significant impairment of liver function. Clinically and experimentally (22) well developed hepatic cirrhosis is accompanied by the development of varices and ascites. The sections of the spore injected livers demonstrated that hepatic cirrhosis did not develop in spite of occlusion of the intrahepatic portal radicles by the spores. An intrahepatic collateral circulation such as has been described has not been previously reported so far as is known. About three-fourths of the liver cells

seen in the autopsy sections of the spore injected dogs were histologically normal.

The liver in pernicious anemia(6) grossly is a pale rusty color and not obviously enlarged. The cut surface is dry and shows rusty brown lobules with yellow centers. Sections show that the brown in the periphery of the lobule is due to the deposition of iron; the pale centers show fatty degeneration.

The experimental findings in this attempt to produce a macrocytic hyperchromic anemia by the intraportal injection of lycopodium spores in the dog show that the damage which can be produced by this method is insufficient to affect measurably the physiologic storage activity of the liver for the anti-pernicious anemia principle. The failure of the method probably is due to the reserve of liver function and to the regenerative properties of liver tissue.

Of the three spore injected dogs saved for future examination, one died on the 208th day after the injection of lycopodium spores from an unknown cause before counts were made. The other two showed normal blood pictures when examined 271 and 334 days after the injection of lycopodium spores.

SUMMARY:

1. The average values and range of variation for erythrocytes, hemoglobin, per cent packed cells, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocytes, and

leukocytes from 63 counts on 19 normal dogs and from 48 counts on 12 normal dogs are given. Variations definitely in excess of the technical error of 2.5% are normally found in counts seven days apart.

2. Lycopodium spores in a 1% suspension in physiological sodium chloride solution were introduced into the intrahepatic radicles of the portal vein of four male and four female dogs with the hope of injuring the function of the liver in hematopoiesis sufficiently to produce a macrocytic hyperchromic anemia. Two male and two female dogs were similarly injected with physiological sodium chloride solution to serve as operated control animals.

3. The eight spore injected dogs throughout a period of nine weeks gave no evidence of the development of any type of anemia as seen by the unchanged erythrocyte counts, hemoglobin values, per cents of packed cells, mean corpuscular volumes, mean corpuscular hemoglobins, mean corpuscular hemoglobin concentrations and leukocyte counts. Further, there was no evidence of damage to other functions of the liver as studied by the Rose Bengal dye excretion test and blood chemistry determinations.

4. Liver extract in clinically adequate amounts produced no change in the blood constituents studied, including the reticulocytes, in either the spore injected or operated control groups of dogs.

5. Autopsy examination of the spore injected dogs showed essentially normal gross findings. Microscopic study of sections of the livers of the spore injected dogs showed that the lycopodium spores were embedded in hyaline fibrous tissue in the portal spaces, and that fully three-fourths of the liver tissue was histologically normal.

6. This attempt at the production of a macrocytic hyperchromic anemia in the dog, therefore, was unsuccessful.

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