"THE EFFECT OF LIVER AND VENTRICULIN ON BLOOD AND ENDOCRINES IN SECONDARY ANAEMIA".

by

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INTRODUCTION.

During the last few years several observers have studied the effects of liver feeding in Secondary Anaemia. Observations have been both experimental and clinical. The animals used for experimental purposes were mostly dogs, rabbits and rats. The consensus of opinion is that liver is of value in the treatment of anaemia secondary to haemorrhage. The references are too numerous to quote in full. To mention only a few, Robscheit Robbins, Whipple and their collaborators (1) have in 1929 observed that "various fractions and residues prepared from beef liver, when fed to dogs made anaemic by bleeding, were all found to contain material potent in increasing the haemoglobin and red-cell content of the blood." Wilkinson (2) in 1931 reports that "Some improvement has been noted in cases of Secondary Anaemia following haemorrhage, gastrectomy and gastro-enterostomy but the results were much increased with the addition of Iron". Dr J. Vaughan (3) in the same year found that "whole liver is of value in treating the Anaemia Secondary to haemorrhage". Murphy and Power (4) in 1929/

1929 have found liver to be effective in Secondary Anaemia. The present work was carried out to observe firstly the effects of liver feeding on blood and endocrines on cats rendered anaemic by bleeding; and secondly the effects of ventriculin feeding on the same tissues in anaemic cats and to contrast the relative value of the two treatments. The results here reported include experiments on forty adult female cats, ten of which were used as controls in that they were not treated with liver or ventriculin. The animals ranged in weight from 2 to 3.5 kilos; the average weight being 2.75 kilos.

TECHNIQUE.

Cats were used in all the experiments. They were fed once daily on a normal diet consisting of milk, 340 cc., and bread, 140 grams with, on alternate days, 140 grams of boiled fish for four weeks. By this time all the animals were well accustomed to the diet, laboratory conditions and routine. The weights of the animals were taken bi-weekly. Samples of blood were withdrawn from the ear vein weekly to obtain normal figures for cell enumeration and haemoglobin estimation. Blood smears were also taken weekly for differential and reticulocyte counts. The total blood volume was calculated according/

according to the results obtained by D.T. Harris (5) which is 4.8 per cent of the body weight by the bleeding method, also according to Erlanger's (6) vital red method which gives 6.2 per cent of the body weight.

At the end of the fourth week anaemia was produced by repeated bleedings from the ear vein of the cat as follows:- The cats were bled at three day intervals until the haemoglobin content was reduced to 50 per cent or 40 per cent of its normal level. Following every haemorrhage, after an interval of forty-eight hours, samples of blood were taken for enumeration of cells, haemoglobin determination and also for blood smears for other counts. Three bleedings were done on each animal at the end of which period the haemoglobin was reduced by 40 per cent or 50 per cent of its original level and this was taken to indicate a sufficient anaemia. Each red and white cell count was made in triplicate and for reticulocytes a thousand red cells were counted and the reticulocytes present in that number were ascertained and a percentage struck. The total amount of blood removed to produce anaemia was in each case 3 per cent of the body weight of the animal which was almost two-thirds or one half of the calculated blood volume by the bleeding method and the vital-red method respectively and/

and if that amount did not reduce the haemoglobin to the desired level i.e. 40 per cent or 50 per cent of the original level a little more blood was withdrawn. This anaemia was then maintained as near a constant as possible by small frequent haemorrhages of calculated amounts and testing at two day intervals for haemoglobin. Blood examinations were done forty-eight hours after each haemorrhage.

The animals were then divided into 3 groups; one group received liver, the second ventriculin and the third treated as controls. The liver batch was fed daily with 60 grams of raw beef liver, 80 grams of bread, alternating with 80 grams of boiled fish and milk, 340 cc. each, for four weeks. The ventriculin group was given daily ventriculin, 60 grams, (P.D. & Co. - 10 grams of the powder equivalent to about 120 grams of fresh tissue) boiled fish 80 grams and milk 340 cc. each, for four weeks. Separate controls were used for each group and the animals of this group received instead of raw liver and ventriculin, beef steak 60 grams.

Blood smears were taken for reticulocytes from each of the animals, twenty-four and forty-eight hours after the commencement of liver, ventriculin and beef feeding. The usual blood examinations were done every week thereafter for four weeks. The control and the experimental/

experimental animals were all rendered anaemic. During the bleeding period and the next four or five resting days the cats were on bread and milk diet.

A few cats were not rendered anaemic and some of them were given raw liver, a few were given ventriculin and a few treated as controls. They were fed in exactly the same way as those rendered anaemic and the same technique was followed on these as well.-

Blood examinations, specimens, smears etc.

Bleeding was done as follows:-

The cats were placed in a wooden rectangular box provided with a lid. The box was of just sufficient size to be of absolute comfort to them; an opening at one end of the box allowed the head to emerge comfortably and to be easily controlled by means of an ordinary metal holder such as is used in blood-pressure experiments, the rod being steadied by an assistant. The ear was rubbed with benzole for a minute or two and warmed. The ear vein thus stood out prominently and a very small longitudinal incision was then made with a sharp-pointed knife and blood was collected in a graduated measuring cylinder with an anti-coagulant in it - Potassium oxalate 2 milligrams to each cc. of blood -. The bleedings were done at a uniform rate in controls and experimental animals so that no difference of water or lymph absorption from tissues should occur. Care was also taken not to bleed/

bleed the animals too rapidly lest it might cause a fatal shock. After the required amount of blood was drawn, the bleeding was stopped, vaseline applied and the animal returned to its cage. Forty-eight hours after the first bleeding samples of blood were taken from the ear-vein for haemoglobin estimation, enumeration of cells, reticulocyte and differential counts. A second bleeding was done the day following the blood examinations in exactly the same way. Samples of blood were again taken forty-eight hours after this bleeding. A third bleeding was done the following day and forty-eight hours after this again blood examination routine was done as above. Four or five days after the last bleeding the animals were put on experimental food. Forty cats were experimented upon; some of the cats showed signs of exhaustion after the third bleeding.

Estimations of blood-sugar, total epinephrine content and blood-pressure tracings were also done on the liver, ventriculin-fed and control animals, apart from histological examination of the tissues.

All the connective tissue from the raw liver was removed. The amount required was weighed, minced well, thoroughly mixed with bread, or boiled fish, milk and given. All the animals took this diet with relish. The cats refused to take ventriculin when mixed with bread but they took it well when mixed with boiled fish and a little milk; milk was given separately in another/

another dish to each cat. The beef given to the control animals was also minced and thoroughly mixed with bread or boiled fish.

The average results of the foregoing blood examinations are given in the following tables: all the animals were on the experimental regime for four or five weeks immediately preceding the periods shown in the tables. Samples of blood and small amounts of blood drawn every now and then are not shown in the tables.

For purposes of clearness of comparison of the control, liver-fed and ventriculin-fed animals, the results obtained have been shown in three group-tables. Thus the control group was obtained by averaging ten; the liver-fed 15, and the ventriculin-fed 15, individual tables.

TABLE I./

TABLE I. Control-group. (average of 10 adult female cats).

Date	Diet Food gms. per day.	Food consumed %.	Wt. Kg.	RBC Mill.	WBC per cmm.	Reticulocytes. %.	Hb. %.	Blood Volume cc.		Differential Count.	
3.7.31	Br:140, Milk 340.	100	2.6								
4.7.31	Fish 140; milk 340.	100	2.6	8,10	19,000	-	74	V.R. 161 cc.	T.V. 125	P-58 L-30	E-10 M-2
5.7.31	Bled 30 cc. N.D.	95									
6.7.31	N.D.	100									
7.7.31	Blood examn. N.D.	100	2.6	5,94	19,800	0.2	52	V.R. 161 cc.	T.V. 125	P-51 L-32	E-15 M-2
8.7.31	Bled 25 cc. N.D.	92									
9.7.31	N.D.	96									
10.7.31	Bld.examn. N.D.	100	2.65	4,92	18,000	1	40	V.R. 164	T.V. 127	P-46 L-43	E-14 M-0
11.7.31	Bled 27 cc. N.D.	100									
12.7.31	N.D.	100									
13.7.31	Bld.examn. N.D.	100	2.65	3,94	17,000	2	36	V.R. 164	T.V. 127	P-45 L-43	E-12 M-0
17.7.31	Fish 80, Mince 60, Milk 340 cc.	100									
18.7.31	Bld.for Rets. Mince etc.	100				1					
19.7.31	" "	100				0.75					
24.7.31	Bld. Examn. "	100	2.65	4,96	17,600	0.5	43	V.R. 164	T.V. 127	P-47 L-40	E-13 M-0
31.7.31	" "	100	2.7	5.84	18,000	-	48	V.R. 167	T.V. 130	P-49 L-39	E-12 M-0
7.8.31	" "	100	2.7	6,80	18,400	-	54	V.R. 167	T.V. 130	P-54 L-36	E-10 M-0
15.8.31	" "	100	2.7	7,60	18,800	-	62	V.R. 167	T.V. 130	P-58 L-32	E-10 N-0
17.8.31	Killed.										

N.D. = Normal diet.
V.R. = Vital Red Method
T.V. = True Value (bleeding method).

P = Polymorpho-nuclears.
L = Lymphocytes.
E = Eosinophils.
M = Mononuclears.

TABLE II. Ventriculin-group (average of 15 adult female cats.)

Date.	Diet Food gms. per day.	Food consumed %.	Wt. Kg.	RBC. Mill.	WBC. per cmm.	Reticulocytes. %	Hb. %.	Blood Volume cc.		Differential Count.	
7.11.31	Br:140 Milk 340.	100	3.35	8,07	18,000	-	72	V.R. 208	T.V. 161	P-53 L-34	E-11 M-2
8.11.31	Fish 140 Milk 340	100									
9.11.31	Bled 35 cc. N.D.	96									
10.11.31	N.D.	100									
11.11.31	Bld.exam. N.D.	100	3.35	5,88	18,400	0.5	56	V.R. 208	T.V. 161	P-46 L-35	E-19 M-0
12.11.31	Bled 38 cc. N.D.	92									
13.11.31	N.D.	100									
14.11.31	Bld.exam. N.D.	100	3.4	4,00	18,000	1	46	V.R. 211 cc.	T.V. 163	P-49 L-37	E-14 M-0
15.11.31	Bled 30 cc. N.D.	100									
16.11.31	N.D.	100									
17.11.31	Bld.examn. N.D.	100	3.45	3,22	17,800	2	34	V.R. 214	T.V. 167	P-46 L-42	E-12 M-0
21.11.31	Fish 80, Ventr. 60, Milk 340	100									
22.11.31	Ventr: etc. Bld. for Rets.	100				2.5					
23.11.31	" " "	100				3					
28.11.31	Ventr.etc.Bld.examn.	100	3.45	4.96	18,000	3.5	46	V.R. 214	T.V. 167	P-48 L-41	E-11 M-0
5.12.31	" "	100	3.5	6,60	18,200	0.5	60	V.R. 218	T.V. 168	P-50 L-38	E-12 M-0
12.12.31	" "	100	3.5	8,20	19,000	-	68	V.R. 218	T.V. 168	P-55 L-36	E-9 M-0
19.12.31	" "	100	3.5	8,90	19,400	-	76	V.R. 218	T.V. 168	P-58 L-32	E-10 M-0
21.12.31	Killed.										

N.D. = Normal diet.
V.R. = Vital Red.
T.V. = True Value (bleeding method).

P = Polymorphonuclears.
L = Lymphocytes.
E = Eosinophils.
M = Mononuclears.

TABLE III. Liver-group (average of 15 adult female cats).

Date.	Diet Food gms. per day.	Food consumed %	Wt. Kg.	RBC. Mill.	W.B.C. per cmm.	Reticulocytes. %	Hb. %	Blood Volume cc.	Differential Count.
28.10.31	Br: 140. Milk 340.	100	2.5	8,60	18,000	-	70	V.R. 155 T.V. 120	P-57 E-9 L-34 M-0
29.10.31	Fish 140, Milk 340.	100							
30.10.31	Bled 33 cc. N.D.	92							
31.10.31	N.D.	96							
1.11.31	Bld.examn. N.D.	100	2.6	5,85	18,800	P +	60	V.R. 161 T.V. 125	P-53 E-11 L-35 M-1
2.11.31	Bled 25 cc. N.D.	98							
3.11.31	N.D.	100							
4.11.31	Bld.examn. N.D.	100	2.65	4,92	17,700	0.08	46	V.R. 164 T.V. 127	P-46 E-13 L-40 M-1
5.11.31	Bled 22 cc. N.D.	100							
6.11.31	N.D.	100							
7.11.31	Bld.examn. N.D.	100	2.65	3,64	16,600	2	30	V.R. 164 T.V. 127	P-45 E-14 L-41 M-0
11.11.31	Liver 60, Br:80, Milk 340.	100							
12.11.31	Blood for Rets. Liver etc.	100				3.8			
13.11.31	" "	100				4			
18.11.31	Liver etc. Blood examn.	100	2.85	5,88	17,000	5	50	V.R. 177 T.V. 137	P-48 E-10 L-42 M-0
25.11.31	" "	100	3.0	8,20	17,800	2	66	V.R. 186 T.V. 144	P-52 E-10 L-38 M-0
2.12.31	" "	100	3.15	8,98	18,600	P +	74	V.R. 195 T.V. 151	P-57 E-9 L-34 M-0
12.12.31	" "	100	3.2	9,40	18,800	-	82	V.R. 198 T.V. 154	P-58 E-8 L-34 M-0
14.12.31	Killed.								

N.D. = Normal diet.
V.R. = Vital Red.
T.V. = True Value (bleeding method).
P + = Polychromes 1 - 2 in each field.

P = Polymorpho-nuclear leucocytes.
L = Lymphocytes.
E = Eosinophils.
M = Mononuclears.

GENERAL OBSERVATIONS DURING THE ANAEMIC PERIOD IN ALL THE ANIMALS.

The erythrocyte count was reduced in each animal to well below 50 per cent of the normal. The white cells showed an increase of 400-800 cells per cmm. of blood after the first bleeding and then gradually decreased by about a thousand cells per cmm. at the end of the bleeding period. The increase in lymphocytes was between 7-12 per cent above normal. There was only a slight rise in eosinophils. The polymorphs decreased by 7-12 per cent. Polychromes appeared in the blood 12-24 hours after the first bleeding; erythroblasts and reticulocytes after the second bleeding. At the end of the third bleeding the reticulocytes had increased to 2 per cent. The number of erythroblasts in the circulation preceded the reticular response by a few hours; in some cases by a few days. The haemoglobin was reduced by more than 50 per cent of normal. All the cats increased in weight during the anaemic period by about 50 to 100 grams.

RESULTS OF BLOOD EXAMINATION AFTER EXPERIMENTAL FEEDING.

CONTROL ANIMALS.

With the onset of beef-feeding the red cell count in the control animal increased much more slowly than in the liver and ventriculin fed groups:/

groups; the increase being roughly a million cells per cmm. of blood each week (Table I. Control-group). It only reached approximately the pre-existing level 28 days after the low point due to bleeding was established. It was 500 thousand cells short of the normal.

The white cells also increased gradually from 17,000 to 18,800 per cmm. of blood at the end of the fourth week. The original count was 19,000 per cmm. of blood. There was a gradual decrease of lymphocytes from 43 per cent at the end of the bleeding period to 32 per cent at the end of the experiment. The increase in polymorphs was from 2-5 per cent during the feeding period. The differential count was much about the normal at the close of the experiment. There was little or no change in the eosinophils.

The reticulocytes had decreased by 1 per cent at the end of 24 hours and 0.25 per cent at the end of 48 hours after beef feeding. At the end of the first week of feeding it had gone down by another 0.25 per cent and completely disappeared by the end of the second week.

There was only a slight increase of 5-8 per cent in haemoglobin each week and it was only 62 per cent at the end of the fourth week whereas it was 74 per cent originally.

VENTRICULIN-FED/

VENTRICULIN-FED ANIMALS.

A week's feeding with ventriculin showed in the blood a definite gain in the number of erythrocytes as compared with that of the control group. There was an increase of one million seven hundred thousand cells each week for 2 weeks; and the count was more than back to normal in three weeks (Table II. Ventriculin-group). The white cells also increased gradually but steadily and was more than normal at the end of the 3rd week of the experiment. The lymphocytes decreased very gradually from 42 per cent at the end of the anaemic period to 32 per cent at the end of four weeks. There was no distinct change in the eosinophils. The neutrophils increased from 2-5 per cent each week and was back to normal in 3 weeks' time.

The reticulocyte response was not great. There was an increase of 0.5 per cent twenty-four hours after and 1 per cent forty-eight hours after the commencement of ventriculin feeding. Towards the end of the first week of feeding, there was an increase of 0.5 per cent in the number of reticulocytes. In the second week it had decreased by 3 per cent; in the third and fourth week the reticulocytes disappeared completely.

The haemoglobin production in this group increased more rapidly than the controls. There was an increase of 12 per cent the first week, 14 per cent the second week/

week and 8 per cent the following two weeks. The haemoglobin percentage was just below i.e. 68 per cent at the end of the third week and 4 per cent above 72 per cent - the normal-towards the end of the experiment. The regeneration of blood in this group was almost complete at the end of the third week.

LIVER-FED ANIMALS.

Feeding with beef-liver for a week resulted in a remarkable increase of erythrocytes; there was a gain of 2,200,000 in the red cell count each week, so much so at the end of the second week the count was almost back to the normal. The third and the fourth weeks showed a distinct gain over the pre-experimental level. In this group there was a striking difference as compared with the other results obtained with beef and ventriculin feeding. (See Graph I).

The white cells increased steadily and gradually each week reaching almost the normal in two weeks' time; during the third and fourth weeks it was 600-800 cells above the normal. The lymphocytes which had increased from 34 per cent to 41 per cent during the anaemic period remained almost steady during the first week of feeding. It then gradually receded to normal at the end of three weeks. The neutrophils increased/

increased also gradually by 3-12 per cent in three weeks, when it was normal. There was no rise in eosinophils. The differential counts of the animals in the three groups did not show any marked difference.

The reticulocytes increased by 1.8 per cent after 24 hours and 2 per cent after 48 hours of liver feeding; there was an increase of 1 per cent over the percentage just noted, at the end of the first week of feeding. On the whole there was an increase of 3 per cent in the reticulocytes within a week of liver feeding. At the end of the second week it had decreased by 3 per cent from the previous week's increase and disappeared altogether at the end of the experiment. During the third week there were found 1-2 polychromes in each field.

The liver diet tables show a notable difference in haemoglobin regeneration. In the first week there was a remarkable gain of 20 per cent haemoglobin regeneration; in the second week it was 16 per cent above the previous week and the third and the fourth weeks showed an increase of 8 per cent each week over and above the second week. In this group of animals the total haemoglobin regeneration was from 30 per cent to 82 per cent in four weeks of liver feeding. The pre-experimental percentage of haemoglobin was 70 per cent. So the haemoglobin regeneration was 12 per cent/

cent above normal due to liver feeding in four weeks. The total haemoglobin regeneration due to ventriculin feeding for four weeks was from 34 per cent to 76 per cent; the original level was 72 per cent, whereas the control animals showed an increase from 36 per cent - the anaemic level - to 62 per cent at the end of four weeks of beef-feeding; the initial level for this group was 74 per cent. (See Graph II).

The return of the number of red blood cells and haemoglobin percentage to the value noted before any loss of blood had been sustained by the cats were the criteria used in judging regeneration.

As to morphological changes, polychromatophilia was especially noticeable. This type of cell appeared in 12-24 hours after haemorrhage and gradually increased in number reaching the highest point on the third or fourth day, disappearing again in the course of a week or two after experimental feeding. These polychromatic red cells were larger than the red cells so the blood has appearance of a remarkable anisocytosis. Normoblasts, basiphilic punctates and reticulocytes were also seen in the early stages of anaemia but they were few in number (See tables for reticulocytes). Poikylocytosis was also noted.

In the blood smears of the normal cats amongst the leucocytes were found some cells resembling polymorphs/

polymorphs which were larger than the eosinophils, with lobed nuclei, like the polymorphs and the cytoplasm of these cells contained granules coarser than those of neutrophils, finer than eosinophil granules and these took on a neutral strain. This kind of cell was very very few. The lymphocytes in cat's blood were found to be of three types - large, medium-sized and small -. The protoplasm of some of these lymphocytes was hyaline but some had a granular protoplasm which stained a much deeper blue than the hyaline cells. There were also a few cells with round nucleus, larger than the large lymphocytes which contained coarse blue granules in the cytoplasm, which was also basiphilic.

Differential counts were done by the "Four-field" meander method of Victor Schilling (7) on margin-free smears. Reticulocyte Counts were done in smears - permanent preparations. Perfectly clean slides were previously treated with Brilliant Cresyl Blue (0.3 per cent alcoholic solution); smears were taken in the usual way and counter-stained with May and Grünwald stain followed by Giemsa. The smears were fresh and unfixed. Cat's blood takes a much longer time to stain than human blood. The fresh unfixed smears were covered with May and Grünwald Solution for ten minutes, diluted with an equal amount of distilled water and allowed to stain for twenty minutes. It was then/

then counter-stained with freshly prepared Giemsa by substituting diluted May and Grünwald solution with Giemsa Stain without previous drying for thirty minutes. They were then rinsed in distilled water and left in it for another 10 minutes, rinsed again, dried and examined. Other blood smears for differential counts were also stained with May and Grünwald followed by Giemsa in the same way.

HISTOLOGICAL TECHNIQUE.

At the end of the fourth week of experimental feeding the animals were killed and all the endocrines, liver, spleen and lymph glands were removed for histological purposes. Sections of liver, spleen, lymph glands, pancreas and ovaries were stained with Delafield's Haematoxylin and Eosin and by other methods mentioned later. All the sections were cut at 4 μ except the thyroids which were cut at 7 μ . Pituitary Sections were stained with May and Grünwald solution. Supra-renals were fixed with osmic vapour according to Cramer's (8) method. The thyroids were stained with aniline blue and orange G by the modified Azan Method using a slight difference of technique which has been found to result in a sharper staining reaction of the colloid. The sections were taken down to water fixed in 1 per cent aqueous phospho-molybdic Acid for 5 minutes, washed well in water, stained with Aniline blue and Orange G (Mallory) (9) for/

for 20 minutes and before dehydrating at this stage as in the original technique they were washed in running water for 20 minutes, then dehydrated quickly in absolute alcohol, cleared in xylol and mounted in Damar. The spleen was also stained with Leishman, the lymph glands with Leishman, Heidenhain's Haematoxylin with Saffranin, Azan, and May and Grünwald followed by Giemsa. Pancreas sections were also stained with May and Grünwald and Giemsa. Liver was also stained for fat and glycogen.

RESULTS OBSERVED IN THE TISSUES.

I. BLOOD CHANGES IN:-

(A) Control Cat.

The red cell count which was reduced by 50 per cent of its normal, as a result of anaemia production, increased gradually after feeding with beef. The leucocyte count increased during the anaemic period and then slowly receded to normal at the end of the fourth week. The 'Hb' content which fell by 50 per cent during the anaemic period very gradually improved during the experimental feeding and did not reach the pre-experimental level at the/

the end of four weeks. There was no definite increase in the reticulocytes which were present in the smears as a result of anaemia. There were also found a few lymphocytes with their nuclei undergoing mitotic division during the anaemic period but after feeding with beef none were found.

(B) Ventriculin-fed cat.

The erythrocyte count increased more rapidly after the onset of ventriculin feeding than in the control animals and reached its normal or a little more than normal at the end of the fourth week of feeding with ventriculin. The increase in the percentage of haemoglobin was also more marked than the controls and reached almost its normal level at the end of the third week, but towards the close of the experiment it was slightly above the original percentage. The increase in reticulocyte percentage was very little. The lymphocytes with nuclei undergoing mitotic division were found to be slightly increased after ventriculin feeding.

(C)/

(C) Liver-fed cat.

A week's feeding with raw beef liver increased the red-cell count much more rapidly than in the controls or even the ventriculin-fed cats. There was a gain of over two million cells each week, after the production of anaemia, with raw liver feeding. At the end of the experiment the red count was nearly a million above the original level. The 'Hb' production also was more marked. Liver feeding gave a substantial gain of 20 per cent Hb. the first week and 16 per cent the next week and it was well over the initial level at the end of the experimental feeding. The reticulocytes did not increase beyond 3 per cent and completely disappeared during the third and fourth week of feeding. As a result of neutropoenia there was a relative lymphocytosis during the anaemic period which gradually reached the normal in three weeks' time. The lymphocytes with nuclei undergoing mitotic division were found to be definitely increased but not enough to strike a percentage.

II. LIVER CHANGES IN:-

(A) Control-cats. (Fig. IX)

1. The cells around the intra-lobular vein in many of the hepatic lobules had few granules in them but contained fat globules.
2. Those towards the periphery were filled with granules and had little or no fat globules.

(B) Ventriculin-fed cats. (Fig. X)

1. More or less normal in structure apart from a slight increase in the stored glycogen in the periphery of some of the lobules.
2. Cells contained fewer fat globules than the controls.

(C) Liver-fed cats. (Fig. XI)

1. The cells around the intra-lobular veins in many of the hepatic lobules were filled with granules and those at the periphery were loaded with glycogen.
2. The sections on the whole presented a striking difference compared to the ventriculin-fed or control group livers.

3./

3. There were markedly fewer fat globules in the cells than in the control; or none at all.

III. SPLEEN.

(A) Control-group.

Few giant cells were found; otherwise normal.

(B) Ventriculin group.

1. No appreciable difference.
2. Capsule thinner than in control.

(C) Liver group.

1. No appreciable difference.
2. Capsule thinner than in control.

IV. LYMPH GLAND.

(A) Control.

1. Lymph nodes were packed with granules.
2. Germ centres were of normal size.
3. There were found in the connective tissue, the peripheral lymph channels and medullary portion, a few cells which were slightly larger than the other cells with round nuclei, its cytoplasm staining deep pink containing coarse acidophil granules.

(B) Ventriculin group.

1. The lymph nodules appeared less compact and contained fewer cells than in the control tissue.
2. The germ centres were larger than normal.
3. In the reticulum throughout the gland both in the peripheral channels and in the medullary strands were found cells which were large with round nucleus and darkly stained pink cytoplasm containing large pink granules. These were found to be definitely increased.

(C) Liver group.

1. In the lymph nodules the cells were so few and scattered that the nodules appeared indistinct.
2. The germ centres were very large in proportion to the total size of the nodule.
3. In the connective tissue, in the lymph-channels, in the germ centres and in the medullary portion were found cells which were slightly larger than lympho-cytes. They had clear spherical nuclei/

nuclei; the cytoplasm taking a deep acid stain and they contained large coarse acidophil granules. These were greatly increased in number especially in the peripheral lymph channels.

V. OVARIES.

(A) Control-group. Normal.

(B) Ventriculin-group. No appreciable difference.

(C) Liver-group. No obvious change to be noted.

VI. PANCREAS.

(A) Controls.

1. The cells of the secreting acini were normal.
2. Some of the islets of Langerhans were small and some large.
3. They contain more 'β' cells than 'α' cells.

(B) Ventriculin-fed cats.

1. The cells of the secreting acini were smaller than in the control.
2. Some islets were small and some large.
3. Normal proportion of 'α' and 'β' cells.

(C)/

(C) Liver-fed cats.

1. The cells of the secreting acini were small.
2. Many large islets and a few small.
3. Some of the islets contained more oxyphil than basiphil cells.

VII. PITUITARY.

(A) Control-group. (Fig. XII)

1. The anterior lobe contained more oxyphil than basiphil cells.
2. Capillary spaces were normal.
3. Pars tuberalis normal.
4. Posterior lobe normal.

(B) Ventriculin-group. (Fig. XIII)

1. Pars anterior contained more basiphil than oxyphil cells as compared with the controls.
2. Capillary spaces were slightly dilated.
3. The cells were smaller than normal.
4. Posterior lobe normal.
5. Pars tuberalis normal.

(C) Liver-fed cats. (Fig. XIV)

1. The anterior lobe contained far more basiphils than eosinophils.

2./

2. The capillaries were dilated.

3. The cells were definitely smaller than normal.

4. Posterior lobe showed no change.

5. Pars tuberalis appeared normal.

The nuclei of some of the basiphil and oxyphil cells of Pars anterior were undergoing mitotic division. This change was noted in all the three groups.

VIII. SUPRA-RENALS.

(A) Control-group. (Fig. XV)

1. Cortex - normal.

2. Medulla.

(a) Black type of cells preponderated.

(b) The white variety of cells contained one or more globoid bodies.

(c) The majority of the cells of both types were filled with granules; some contained few granules.

(d) Most of the nuclei of these cells were clear and turgid.

(e) There were found dark brown bodies of different shapes - mainly oval and cup-shaped - in both types of cells; probably shrunken nuclei.

(f) The central vein contained a few greyish granules of adrenaline.

(B) Ventriculin-group. (Fig. XVI)

1. Cortex. Lipoid was distributed more or less throughout the cortex.

2. Medulla.

(a) Most of the cells were of the white variety.

(b) Some of the cells were packed with granules and some contained very few granules as compared with the controls.

(c) The central vein contained greyish granules of adrenaline.

(d) There were some other large granules in the cytoplasm of both dark and light cells which were of varying shapes as mentioned above - and were stained dark brown. These were probably collapsed nuclei.

(e) The capillary spaces were not dilated.

(C)/

(C) Liver-group. (Fig. XVII)

1. Cortex.

(a) Lipoid scattered throughout the cortex.

(b) Zona reticularis contained dilated blood vessels loaded with corpuscles.

2. Medulla.

(a) The white variety of cells very definitely predominated.

(b) These cells did not contain nearly as many granules as the control and ventriculin-fed animals. Globoid bodies were present in their cytoplasm.

(c) The protoplasm of some of the black type of cells had a laked or glazed appearance.

(d) The nuclei were shrunken, cup-shaped or oval in most of the cells in both types - and were stained brown resembling in size and in appearance a red blood cell.

(e) The central vein and its tributaries were filled with greyish granules of adrenaline with a few red blood cells amongst them.

IX. THYROIDS.

(A) Control cats. (Fig. XVIII)

1. The vesicles were large and were entirely filled with colloid.
2. The colloid was stained orange in most of the vesicles.
3. The inter and intra-alveolar capillaries were collapsed.
4. Most of the vesicles were lined by cubical epithelium.

(B) Ventriculin-fed cats. (Fig. XIX)

1. The vesicles were smaller than normal.
2. The colloid in many of the vesicles was shrunken and stained blue and in some cells it was stained orange.
3. The lining epithelial cells were mostly cubical.
4. The inter and intra-alveolar capillaries tend to be collapsed though a few here and there were dilated.

(C) Liver-fed cats. (Fig. XX).

1. Most of the vesicles were very small and were irregular in outline.
2. The colloid was very much shrunken in the majority of the vesicles and was chiefly/

chiefly stained blue with aniline blue; only a very few vesicles showing yellowish or orange colloid. Some sections contained all blue colloid.

(3) Most of the vesicles were lined by cubical epithelial cells taller than those found in the control and ventriculin-fed animals.

(4) The inter and intra-alveolar capillaries were dilated.

X. PARATHYROIDS.

(A) Control-group - normal.

(B) Ventriculin-group. No appreciable difference.

(C) Liver-group. - No change of note.

Blood-sugar estimations.

These were done according to the method of Hagedorn and Jensen (10).

The average result of the various blood-sugar estimations of the cats was:-

(A) Control-cats.

122 milligrams per cent.

(B) Ventriculin-fed group.

116 milligrams per cent.

(C) Liver -fed group.

118 milligrams per cent.

The/

The slight differences in the three averages might be taken to be within the limits of experimental error.

Epinephrine determinations.

Chemical estimations of adrenaline, were carried out by the method of Folin, Cannon and Denis (11). The weights of the animals used for this experiment ranged between 2.45 and 2.5 kilograms. The total quantity of adrenaline of the normal cats, anaemic control cats, ventriculin-fed and liver-fed cats was determined separately.

The weight of the suprarenals of all the three groups of cats was between 0.34 gram and 0.39 gram. The total epinephrine in the normal, anaemic control and ventriculin group was almost the same; there was a definite decrease in the liver-fed group as shown in the table:-

Animals.	Weight of glands gram.	Total epinephrine milligram.	Epinephrine per gram of gland milligram.
Normal	0.36	0.7776	2.16
Anaemic) Control)	0.34	0.7276	2.14
Ventriculin) group)	0.34	0.7242	2.13
Liver group	0.39	0.4719	1.21

From/

From the above table it will be seen that there was only a difference, in the total epinephrine, of 0.05 milligram between a normal cat and an anaemic control; 0.0034 milligram between the control and ventriculin groups.

Practically little significant difference was found in the total epinephrine content of these animals. The liver group showed a definite decrease of 0.3057 milligram in the total adrenaline and 0.95 milligram per gram of gland; from those of a normal cat. The difference between the liver and ventriculin group was 0.2523 milligram in the total and 0.92 mg. per gram of gland; and that between liver and control group was .2557 mg. total and 0.93 mg. of adrenaline per gram of gland. This decrease of total epinephrine in the liver-fed group might be taken to indicate activity.

EXPERIMENTS IN WHICH ADRENALINE CONTENT OF GLANDS DETERMINED BY BIO-ASSAY.

MATERIAL AND METHODS.

Three batches of adult cats were used. Each batch consisted of non-anaemic males, non-anaemic and anaemic females. One animal in each batch was used as a control, one was ventriculin-fed and one was liver-fed in the same way as in the other experiments.

At the end of the feeding period the animals were killed, the suprarenals of each animal weighed and extracted with Ringer Locke, the amount of extract being accurately measured.

The bio-assay was carried out as follows: A cat was decerebrated under deep ether anaesthesia and its vagi cut, or, alternatively a cat was anaesthetised with urethane and its vagi cut and carotid sinuses denervated. A blood pressure cannula was placed in the femoral artery of one side and an injection cannula in the vein of the opposite side. When the blood pressure had attained a constant level, known amounts of adrenaline were injected intravenously. The adrenaline of P.D. & Co. was employed. One cc. of this was accurately measured and made up to 50 cc. with Ringer Locke solution. Each cc. of the diluted solution thus contained 1/50 mgm. adrenaline chloride. Two cc., 1 cc., half a cc., quarter cc. of the 1 in 50,000/

50,000 solution were injected at intervals, several injections of each amount being given and the blood-pressure rise recorded in each case. Thereafter known amounts of the extracts from the animals were injected, usually 1 cc. and ½ cc. These injections were repeated several times, further injections of the stock solution of adrenaline also being repeated at intervals. The tracings were varnished and when dry the blood-pressure rises resulting from the different injections were measured. It was found in all cases, that for similar amounts of adrenaline and of extract the blood pressure changes did not differ by more than five or six millimetres except at low dilutions when the pressure rises were small. Averages of the readings were taken and a graph prepared showing on the ordinate rise in blood pressure in millimetres of mercury and on the abscissa, amount of adrenaline in milligrams. From this graph the amount of adrenaline present in a known amount of the particular extracts can be immediately determined.

RESULTS.

In figure V is shown the graph from which the results for batch III are calculated. In all cases the blood pressure rises plotted are averages of several readings though as noted before the individual/

individual rises vary only slightly. It will be seen that one cc. of extract L gave 94 mm. rise corresponding to an adrenaline content of 0.03625 mgm. Thus the total quantity of adrenaline present in the extract was 0.54375 mgm. Now the weights of the glands in this case was 0.4174 gram.; so that the adrenaline content per gram of gland was 1.302 mgms. Similarly with the other two extracts of this batch and also those of the other batches as shown in figures III and IV.

Following is a summary of the results in the three experiments.

Animals.	Batch I Adr: mg. per gm. of gland	Batch II Adr: mg. per gm.	Batch III Adr: mg. per gm.	Average Adr: per gm. of gland.
Control	0.962	0.619	0.6627	0.7479
Ventriculin-fed.	0.505	0.869	0.206	0.5266
Liver-fed.	1.153	0.818	1.302	1.091

The averages of the biological estimations show liver to be the most effective, thus confirming the histological and chemical findings, though the differences between the averages of the two estimations are great, which may be due to greater accuracy of the biological determinations.

DISCUSSION.

The changes in the blood indicate that as a result of feeding with liver and ventriculin there is a regeneration of blood in secondary anaemia produced in cats. Raw liver feeding was found to be more effective than ventriculin. There was no marked reticulocytosis with the experimental feeding. The haemoglobin content, which was reduced by the production of anaemia, rose rapidly and concurrently with liver feeding and reached a level higher than the original. The animals fed with ventriculin also showed a rapid return to the normal haemoglobin percentage but not so marked as with liver feeding; whereas in the control animals the haemoglobin content increased very gradually and even at the end of the experimental period had not reached the normal 'Hb' percentage.

The red blood cells of the liver-group had remarkably increased after four weeks of feeding. In the ventriculin group the red cell count was a little above the pre-experimental level whereas the control group had not reached the original level at the end of four weeks of feeding. There was therefore a definite increase in haemoglobin and red cell count with liver and ventriculin, more with liver than with ventriculin/

ventriculin feeding.

The blood smears showed poikylocytosis and anisocytosis which are regeneratory phenomena; since these were not found in normal animals it might be considered as an evidence of an excessively hurried regeneration. Polychromasia that was noted 12-24 hours after the first bleeding can also be considered to indicate an active regeneration of normal type - Piney (12).

The nuclei of some of the lymphocytes were found to undergo mitotic division; this was more evident and increased in the liver-fed group. The ventriculin-fed animals also showed a few of these mitotic lymphocytes in the blood smears but less marked. These cells were found in a very small number in most of the blood smears of all animals during the anaemic period but disappeared in the control animals as the blood gradually regenerated. This would show that the lymphoid tissue was stimulated to activity. The very large cells with round nuclei cytoplasm staining blue and containing dark blue coarse granules might be myelocytes and the large cells resembling polymorphs with lobed nucleus and coarse neutrophilic granules in the neutrophil cytoplasm might be hyper-matured polymorphs. These polymorphs with hyper-segmented nucleus increased during the anaemic period in all the animals; only in the control group it gradually disappeared but in the other two groups it disappeared very quickly after feeding. This hyper-segmentation of the nucleus might indicate a premature ageing of the cell or a disturbance of the mechanism for eliminating these/

these aged cells found during the anaemic period.(13) It is interesting to note that a similar type of cell was observed by Piney (12) and W.E. Cooke and C.F. Hill (13) during relapses in pernicious anaemia. The regeneration of blood was much more rapid with liver feeding than with ventriculin as compared with the controls. The blood almost reached its normal within two weeks with raw liver feeding and there was an increase in the weights of the liver-fed group. The fact that several megaloblasts were counted in a few slides shows that in those animals there had been a great marrow disturbance - Gulland and Goodall (14).

The histological changes in the tissues also show that raw liver is more capable than ventriculin of stimulating them to increased activity. The hepatic lobules in the liver of liver-fed animals had a totally different appearance to those of the ventriculin and control groups. The cells of these lobules of liver group had a necrotic appearance - that is they were devoid of most of the granules of a typical hepatic cell -. The presence of increased glycogen in the peripheral cells of most of the hepatic lobules of liver-fed animals might indicate that the liver was active although the blood-sugar revealed no change. The fat globules found in the anaemic control cats might be due to the greater fat content of the beef mince or it might be due to the anaemia/

anaemia that was produced as the same appearance was not found in control cats not rendered anaemic; although a few fat globules are present normally.

The peculiar cells that were found in the peripheral lymph channels, connective tissue and medullary portions of lymph glands and which were increased with liver and ventriculin feeding, more so with the former, might be reticulo-endothelial cells; the sites in which they were found suggest this; if so it would seem that liver and ventriculin feeding stimulate the reticulo-endothelial system in cats rendered anaemic by bleeding. There were also found, in the lymph glands of the liver and ventriculin group, a few cells exhibiting active phagocytosis of red blood corpuscles.

The pituitary of the liver-fed group showed some definite change. The anterior lobe contained far more basiphil cells than the controls. In the ventriculin-fed group the basiphil cells were more than the controls but less than the liver-fed. In thyroidectomised dogs there has been found along with other changes a considerable appearance of oxyphil cells in the Pars anterior of the pituitary (Kojima (15)). Normally there are more oxyphils than basiphils and the fact that there were more basiphils might be argued to indicate excessive thyroid secretion or increased activity of the thyroid. The nuclei of some of the cells of the anterior lobe were found to undergo mitotic/

mitotic division. This increased activity was observed in all the three groups of animals. The production of anaemia might have caused this change as it was better marked in the controls than in the fed animals. Liver and ventriculin feeding had caused stimulation of the basiphil tissue of the anterior lobe of the pituitary as evidenced by their increase under such conditions.

The thyroids of the liver-fed animals were far more active than the ventriculin-fed ones. The thyroids of the control cats presented a normal appearance in that they contained both large and small vesicles filled with colloid which stained orange with aniline blue and orange G. The thyroids of the liver-fed group contained comparatively smaller vesicles, the colloid in most of the vesicles was considerably shrunken and was stained blue. It was always noted that when the vesicles were small and active looking the colloid stained blue. The inter and intra-alveolar capillaries were dilated in the liver-fed group. The lining epithelial cells were almost columnar, nearly always at least tall cubical. All these changes indicate increased activity of the gland. The ventriculin-fed group showed a moderate phase of activity. The vesicles were smaller than those of the control thyroids; some of them contained colloid staining/

staining blue while some colloid stained orange. In some vesicles of the liver and ventriculin groups the colloid was orange surrounded by a blue rim. "This might indicate that the secretion, when in a state to be passed into the blood and used, is alkaline but may become acid when stored for some time". Hewer (16). In others again a few drops of blue were seen in the orange colloid; which would indicate a recent secretion. The same changes occurred in the thyroids of the ventriculin-fed cats but they were not pronounced as stated above. The control animals thyroids contained mostly orange-coloured colloid and a very few with blue.

Staining reactions of colloid as observed by Hewer (16).

	I. Alkaline	Ia. Staining type	II. Acid
Haematoxylin and Biebrich's	Pink	Red	Orange
Weigert's Resorcin - Fuchsin.	Pale purple	Dark purple	Magenta
Aniline blue & Orange G.	Blue	Blue	Orange
Haematoxylin & Congo Red.	Pink	Red	Blue
Haematoxylin & Benzo-purin	Yellow-pink.	Yellow-pink	Blue

Staining/

Staining type I (alkaline) as observed by Hewer (16) was obtained when there was marked activity of the gland and Staining type II (acid) when there was secretory inactivity.

The supra-renals were also stimulated to activity by liver and ventriculin feeding. The cortex of the supra-renal gland of the liver-fed group showed an even distribution of lipoid throughout it and there was marked congestion of the zona reticularis. The ventriculin group also showed these changes in the cortex but to a less extent. Whereas in the control group the lipoid was found in the outer half of the cortex mainly and there was no congestion of reticularis. The medulla of the liver and ventriculin groups showed definite changes. The lipoid in the cortex was removed with turpentine. By the osmic vapour method Cramer (8) has detected in the medulla of normal cats two distinct types of cells - the black and the white variety -, the black type preponderating. Both types of cells were found to contain very fine greyish granules, but the white cells contained in addition to this two or more big coarse black granules in each cell. These were termed globoid bodies and considered to be the precursors of adrenaline.

In the ventriculin-fed animals there was almost an equal distribution of these two types of cells. Some of the cells contained fewer granules than the controls/

controls and some were filled with granules. The central vein contained more granules than the controls and the capillary spaces were dilated slightly. Both types of cells contained along with nucleus and globoid bodies two or more brownish-stained bodies of varying shape; these might be collapsed nuclei found in active adrenals.

In the liver-fed group the medullary cells showed a vacuolated appearance in one part while in some other part of the medulla the cells were filled with granules. The majority of the cells contained few granules and they were fewer in comparison with those found in the ventriculin and control groups. The capillary spaces were dilated. The nuclei of most of the cells were collapsed and the protoplasm of the dark cells presented a laked appearance. All these changes are consistent with increased activity of the gland and liver was found to stimulate the adrenals to greater activity than ventriculin.

The total epinephrine contained in both the glands of the animals taken from the three groups was determined and averaged and from the figures shown in the table there was very little or no change between the controls and the ventriculin-fed animals. The liver-fed animals showed a definite decrease in the total adrenaline content. These facts would indicate that the sympathetic is stimulated much more with raw liver feeding/

feeding than with ventriculin. It must however be noted that the activity or inactivity of the adrenal medulla cannot fairly be based on the amount of granules present in the medulla as the stimulus to secrete adrenaline is also a stimulus to form adrenaline and as Stewart and Rogoff (17) have pointed out that the amount of adrenaline present in the medulla at a given moment is only the difference between the rate at which adrenaline is secreted and the rate at which it is formed. An active gland may therefore be associated with increased or decreased amount of granules in the adrenal medulla.

The black bodies of varying shape which were found in both types of cells of an active adrenal medulla, chiefly in the dark cells might be a transitional stage in the formation of adrenaline possibly from the nucleus.

Blood-pressure experiments also showed liver to be the most effective.

SUMMARY.

1. The average erythrocyte count in normal cats was eight million and white-cell count was 18,000 per cmm. of blood. The percentage of haemoglobin by the method of Haldane was 70 on an average.

2. Cats were used in all the experiments. The cats were rendered anaemic by the removal of approximately one half of the calculated total blood volume by the vital red method or nearly two-thirds of it by the bleeding method; each of which would come to about 3 per cent of the body weight of the animal.

3. Increase in haemoglobin and red-cell count was observed with liver and ventriculin feeding. There was a slight increase in the reticulocytes.

4. Blood-sugar estimations were done but no change was noted in the liver and ventriculin fed animals.

5. Determinations of total epinephrine showed a definite decrease in the medulla of the liver-group; the ventriculin group remained unchanged (was the same as normal).

6. Blood pressure experiments were performed. The tracing for liver-group was the highest.

CONCLUSION.

1. Liver, as tested in these experiments showed a high potency for haemoglobin and red-cell production. The output of new haemoglobin averaged about 16-20 per cent per week. Ventriculin was also found to be potent in these experimental anaemias in cats and it might reach as much as or a little more than one half the potency of raw liver.

2. The thyroids, supra-renal cortex and medulla and anterior lobe of the pituitary were stimulated to greater activity. This was more evident with raw liver than ventriculin feeding.

3. Liver, lymphoid and reticulo-endothelial system appeared to be activated with liver and ventriculin feeding more so with the former than with the latter.

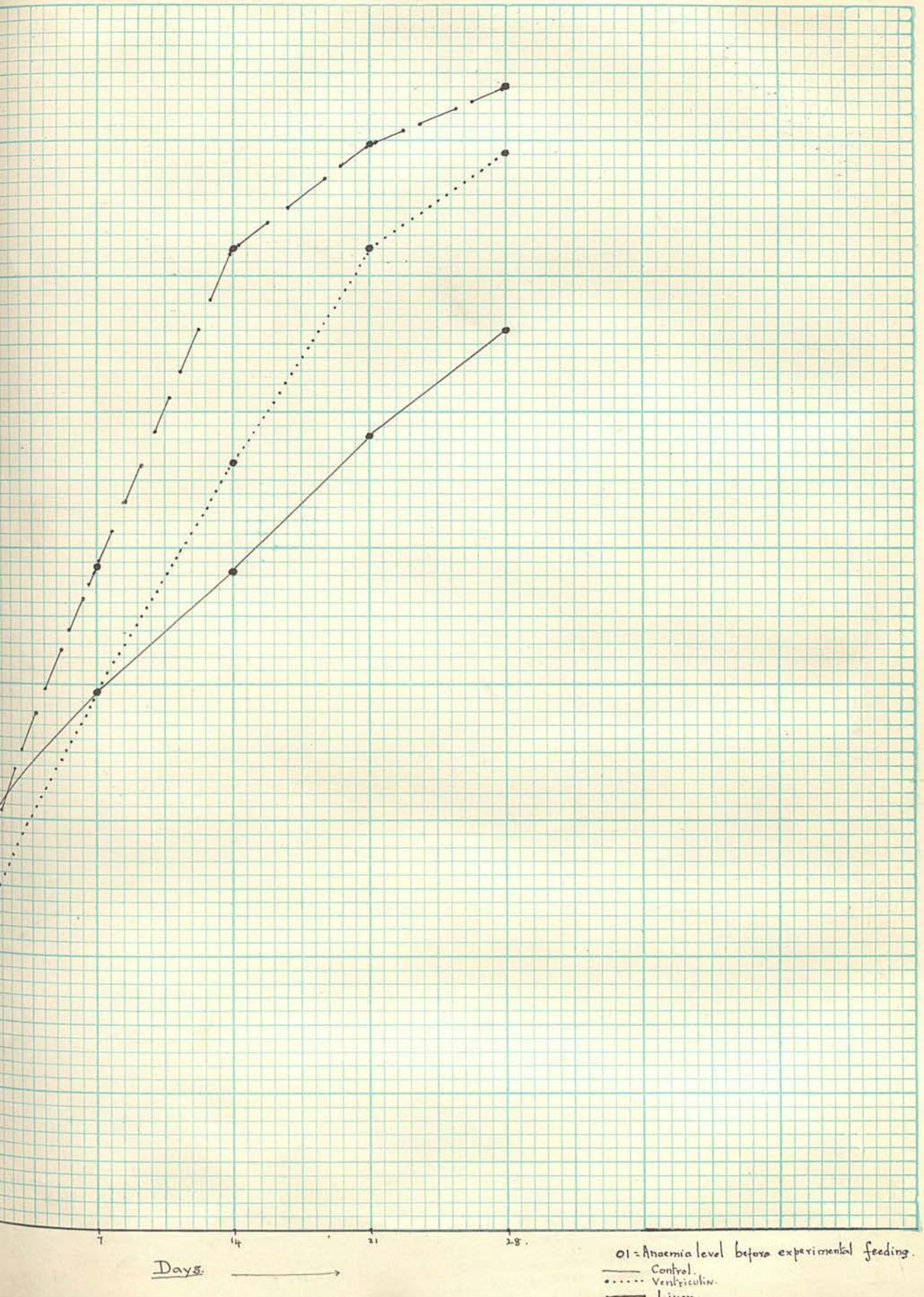
7
14
21
28.
Days.
01 = Anaemia level before experimental feeding.
Control.
Ventriculin.
Liver.

Fig: 1.


7
14
21
28
Days
O : Anaemia level just before experimental feeding.
Control.
Ventriculin.
Liver

Fig: 2.

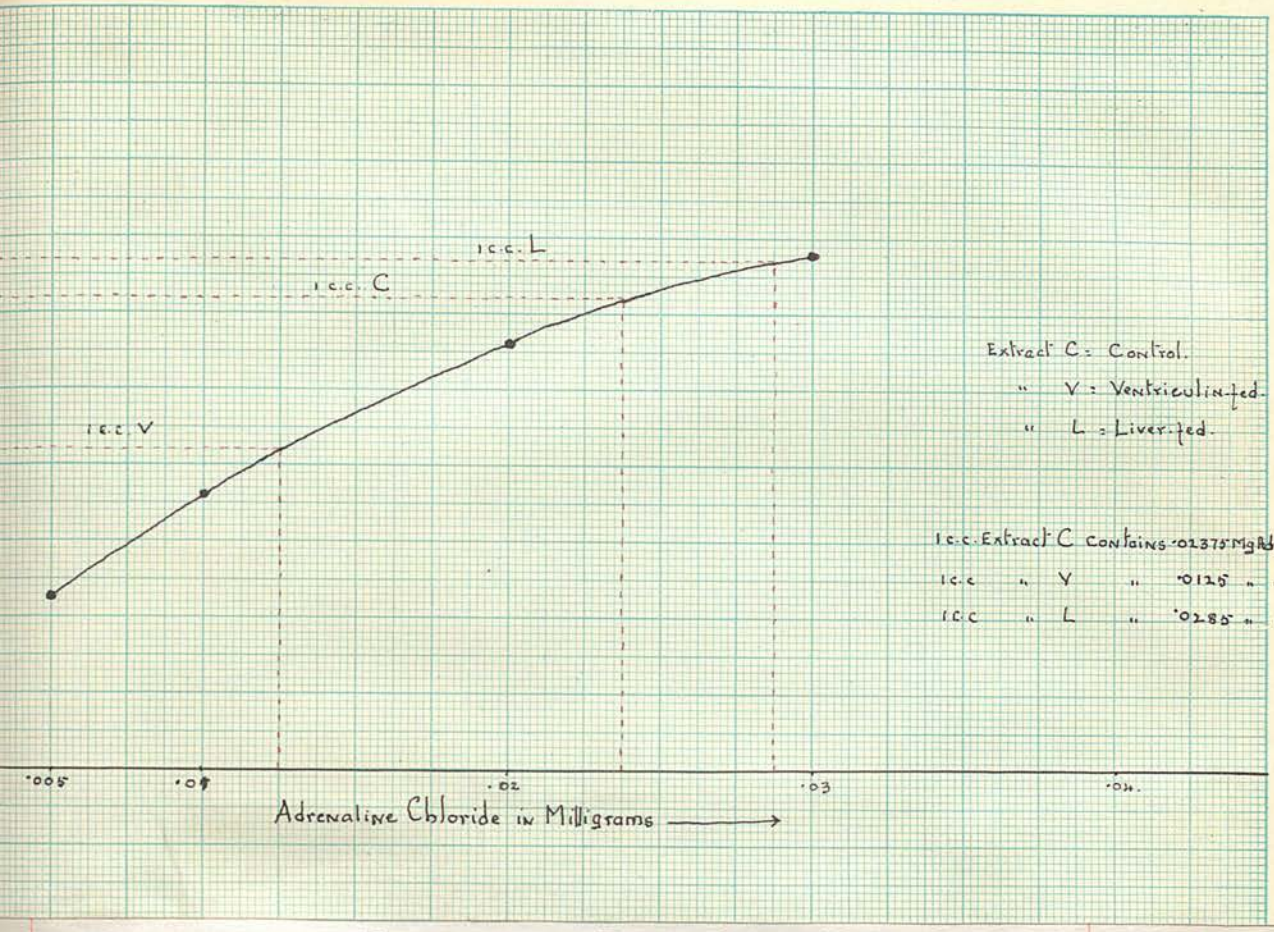

Fig. 3 Batch I

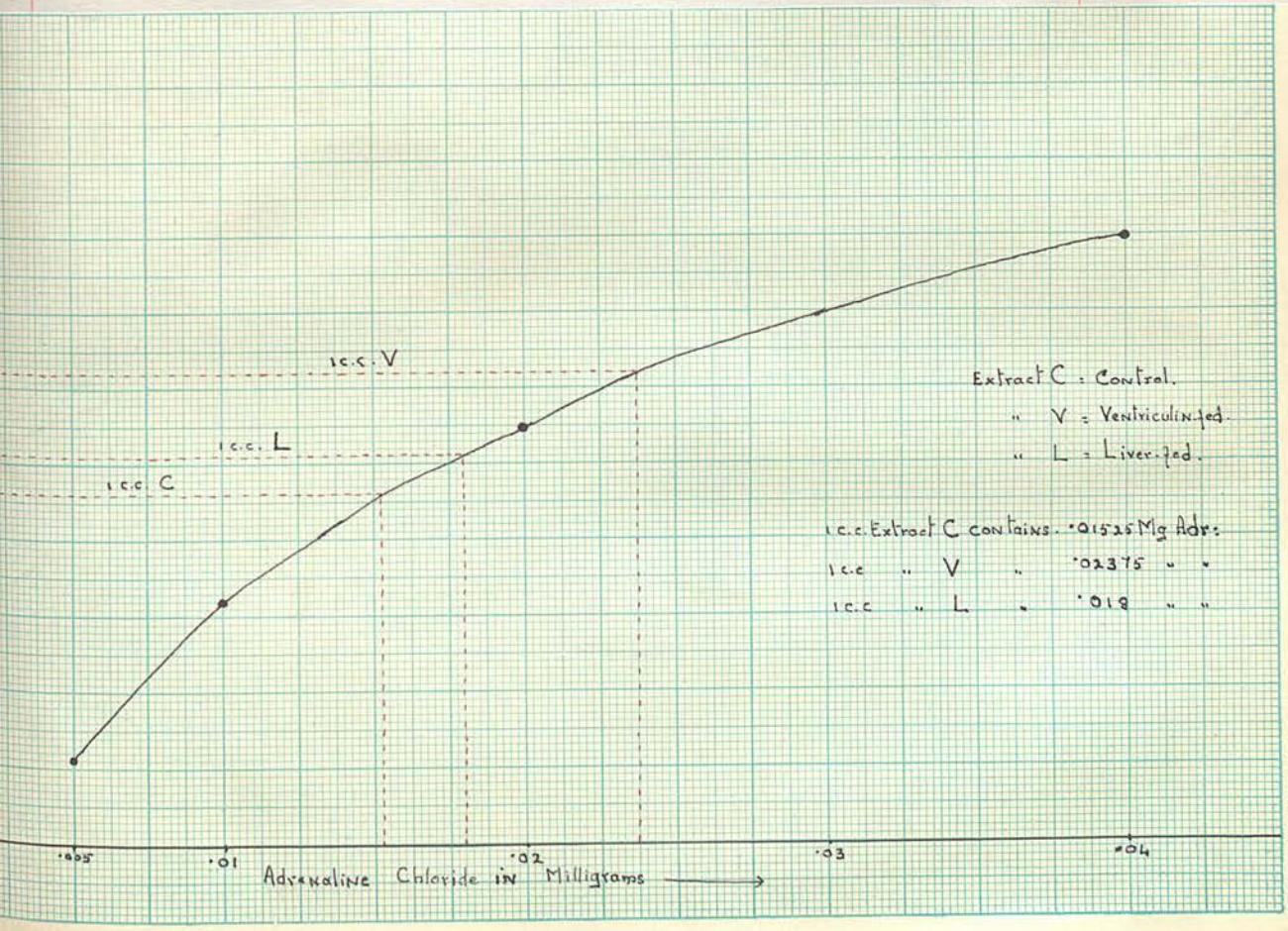

Fig. 4. Batch II

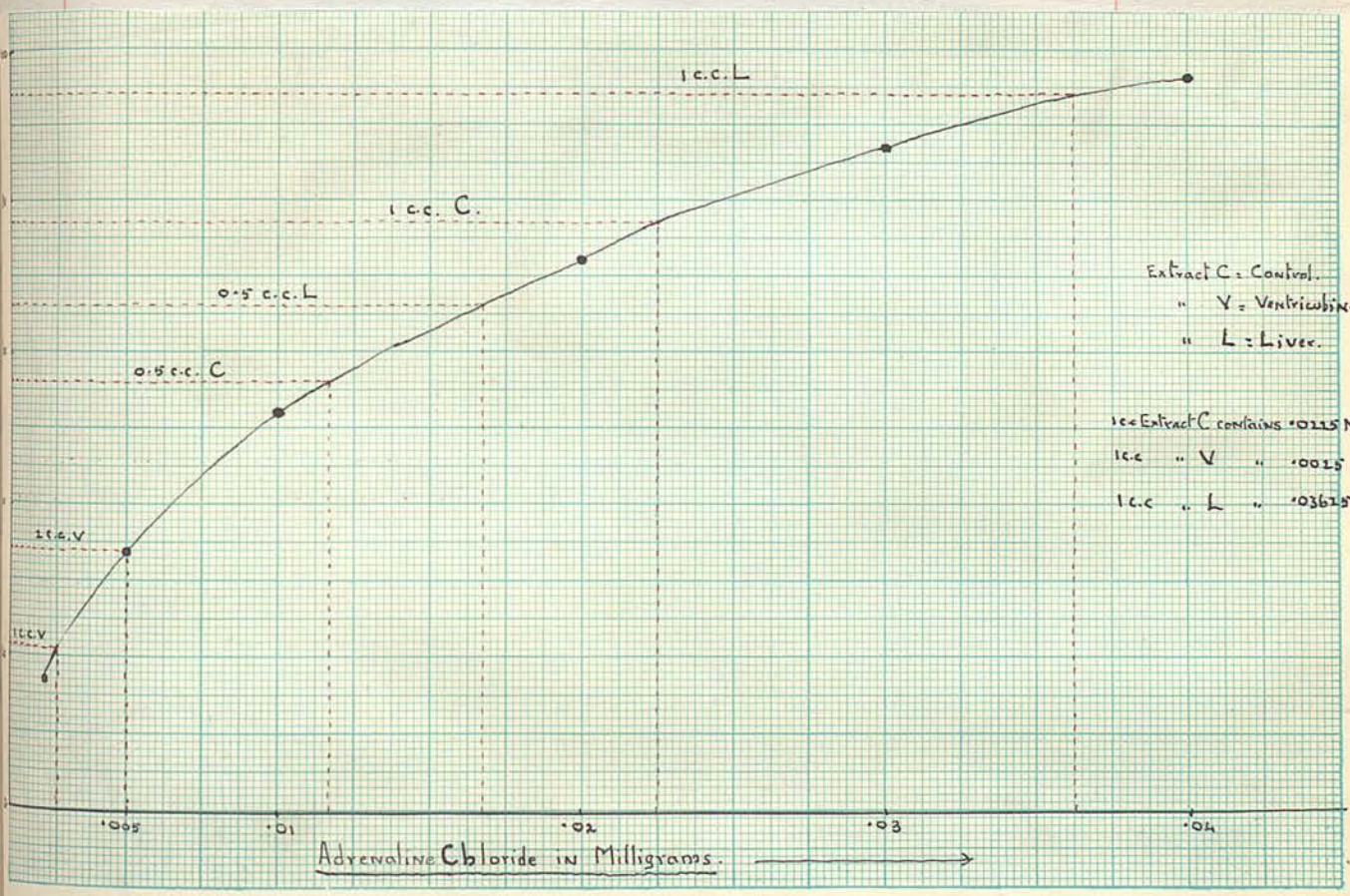

Fig: 5. Batch III

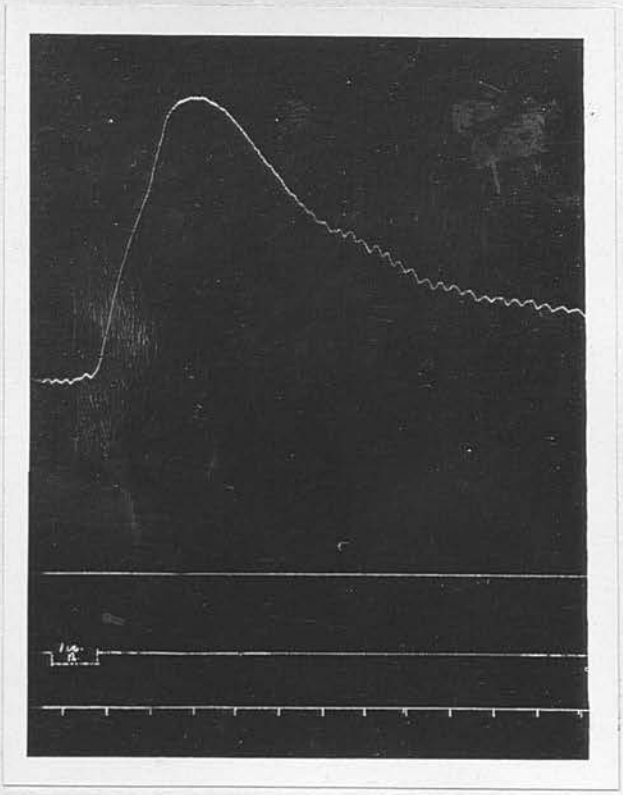

FIG: 6. Blood Pressure : Effect of the Adrenaline extract of a Normal Cat. Batch I

Adrenaline of a Normal Cat 1.c.c at Signal. Blood Pressure rise 62 mm of Hg.

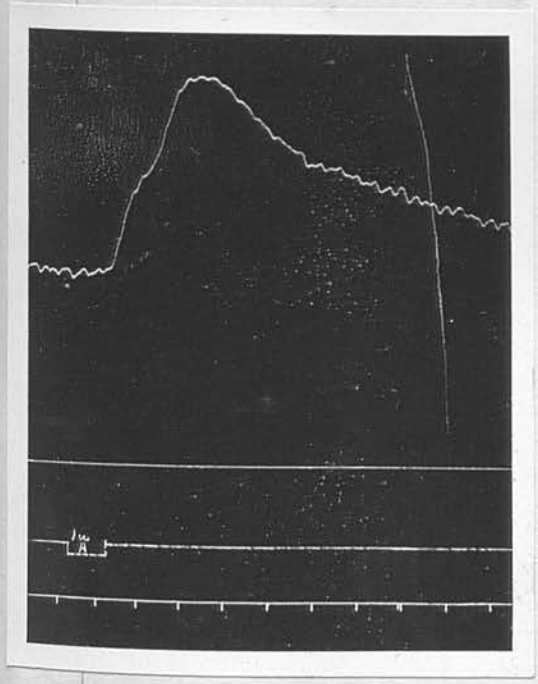

FIG: 7. Blood Pressure : Effect of Adrenaline Extract of a cat fed with Ventriculin. Batch. I

Adrenaline of a cat fed with Ventriculin 1.c.c at Signal. Blood Pressure rise 42 mm of Hg:

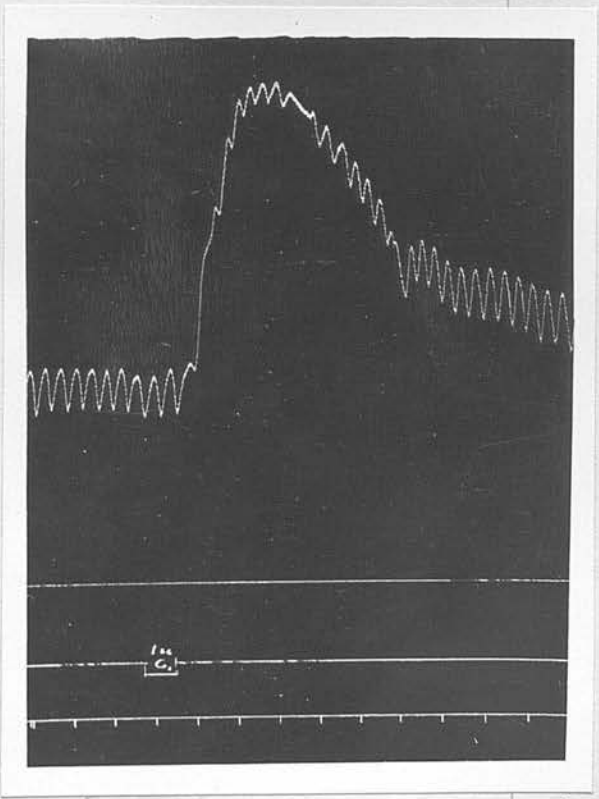

FIG: 8. Blood Pressure : Effect of Adrenaline Extract of a cat fed with raw liver. Batch I

Adrenaline of a cat fed with liver 1c.c. at Signal. Blood Pressure rise 67 mm of Hg.

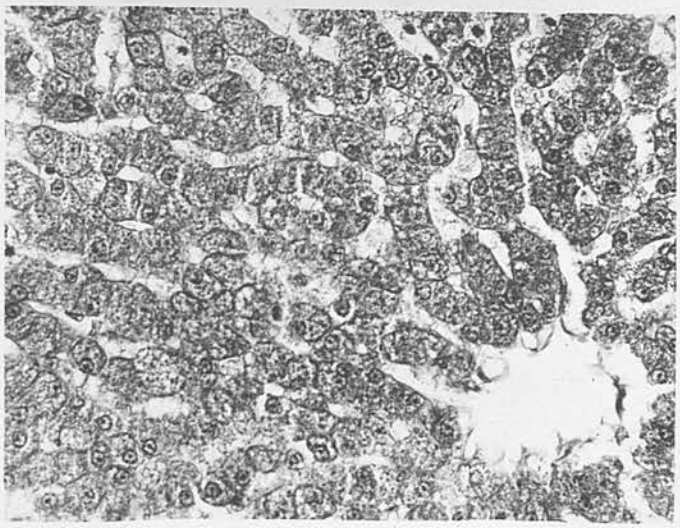

Fig: 9. Liver. Anaemic female Cat. Photo-Micrograph. X 340 diameters. Cells around the vein contain fat globules and fewer granules than those at the periphery. Haematoxylin-Eosin preparation. (control).

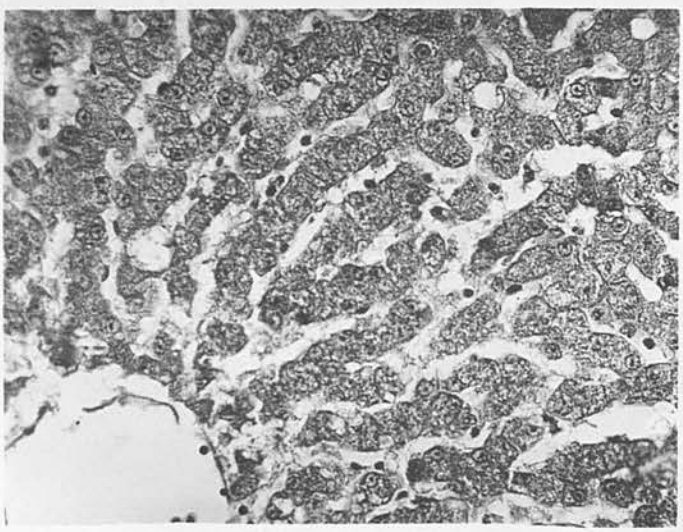

Fig 10. Liver. Anaemic female Cat fed with Ventriculin. Photo-Micrograph. X 340 diameters. Cells around the vein contain more granules than those at the periphery. Haematoxylin-Eosin preparation.

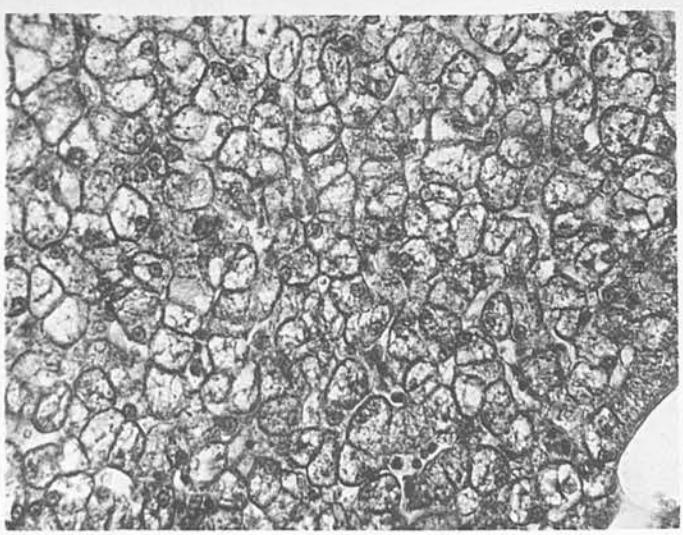

Fig: 11. Liver. Anaemic female cat fed with raw Liver. Photo-Micrograph X 340 diam: Cells around the vein contain few granules while those at the periphery of the lobule are filled with glycogen. Haematoxylin-Eosin preparation.

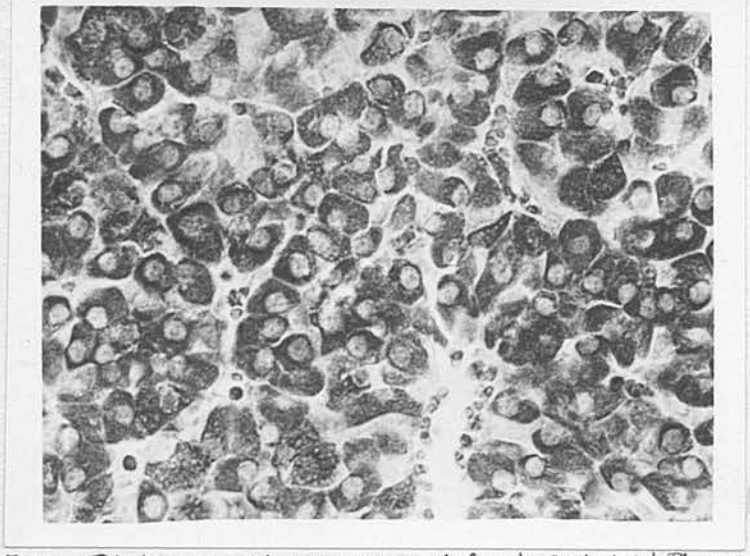

Fig: 12. Pituitary. Pars Anterior of an anaemic female Control Cat. Photo-micrograph. X 550 diameters.
The dark cells are oxyphils; Light cells are basiphils, Clear cells are Chromaphobe.
Blood vessels with a few Corpuscles are seen in the Section. Methylene-blue - Eosin preparation.

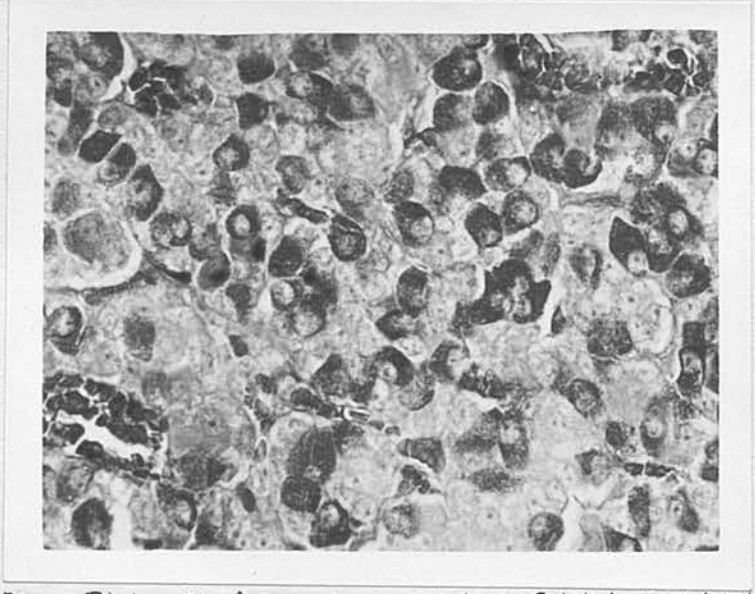

Fig: 13. Pituitary. Pars Anterior of an anaemic female Cat fed with Ventricolin. Photo-micrograph. X 550 diameters.
Oxyphil cells are few and small. Blood vessels are filled with Corpuscles.
Methylene blue - Eosin preparation.

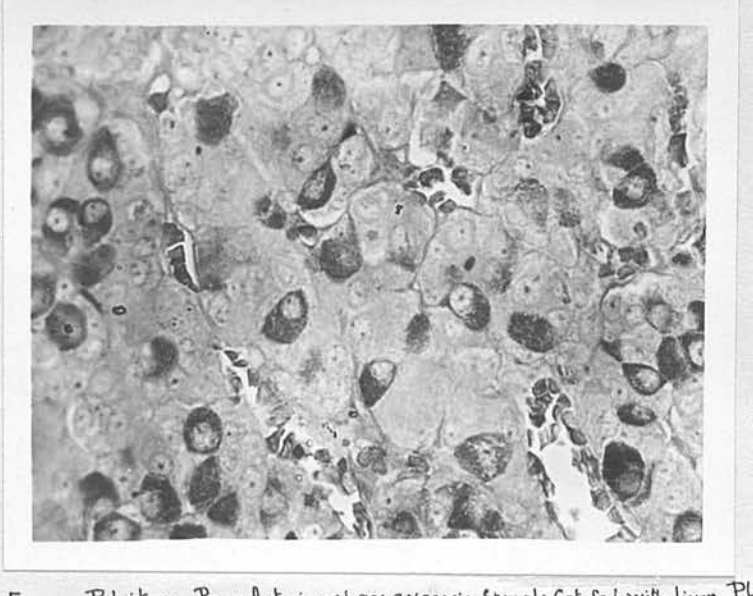

Fig: 14. Pituitary. Pars Anterior of an anaemic female Cat fed with liver. Photo-micrograph. X 550 diameters.
Considerably few oxyphil cells which are also small. There are several blood vessels
With Corpuscles. Methylene blue - Eosin preparation.

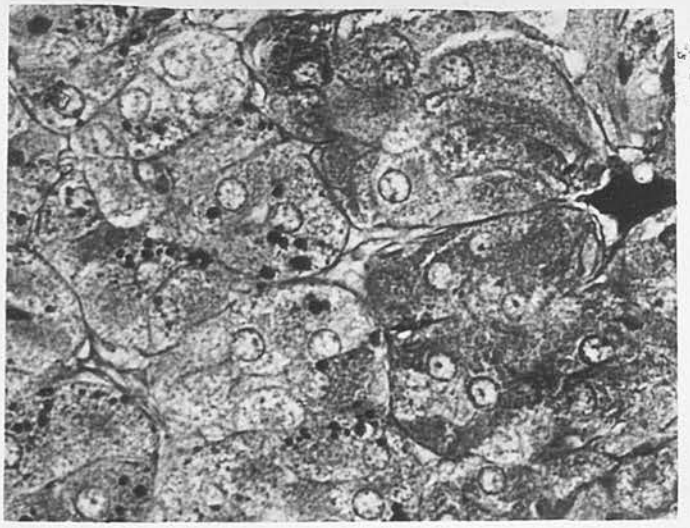

Fig: 15. Adrenal Medulla of an anaemic female Control Cat. Photo-micrograph. X 650 diameters. Some of the cells are black and filled with dark granules; others are light grey also with granules and coarse black granules - globoid bodies - Both types of cells contain clear, turgid nuclei. Osmic vapour fixation.

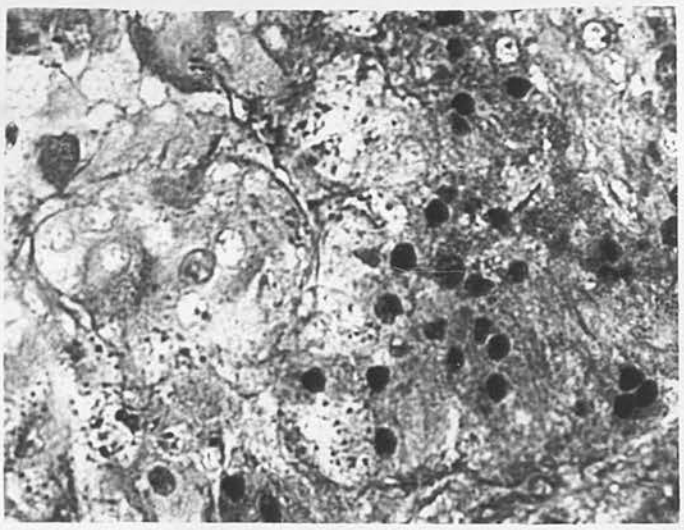

Fig: 16. Adrenal Medulla of an anaemic female Cat fed with Ventriculin. Photo-micrograph. X 650 diameters. Both types of cells contain few granules. Nuclei of most of the black type of cells are darkly stained and are of varying shapes. Globoid bodies are seen in the white cells. Osmic vapour fixation treated with turpentine to remove lipoids.

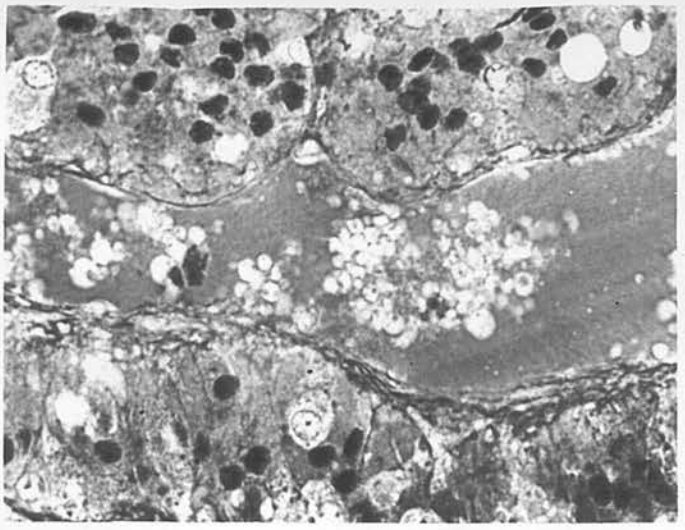

Fig: 17. Adrenal Medulla of an anaemic female Cat fed with liver. Photo-micrograph. X 650 diameters. The Central Vein is seen containing a greyish mass of adrenaline granules, with a few blood cells. Almost all the nuclei are darkly stained. The cells have a glazed appearance and some are vacuolated. Osmic Vapour fixation treated with turpentine.

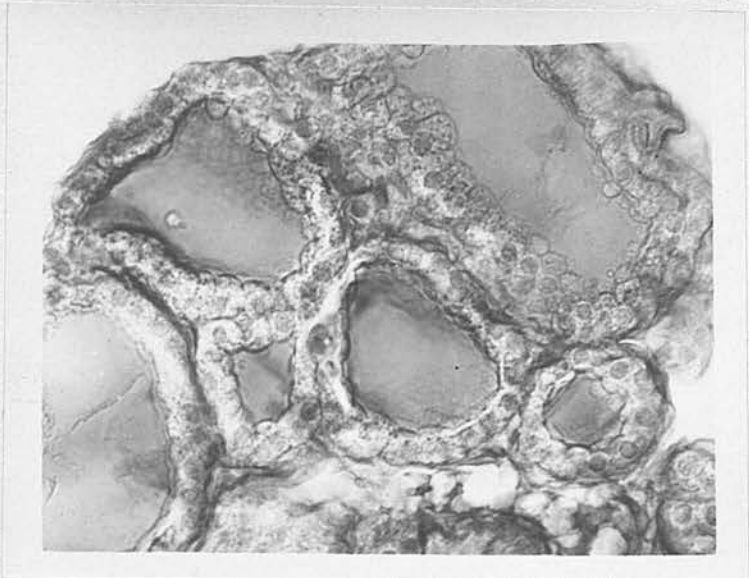

FIG: 18. Thyroid gland of an anaemic female Control Cat. Photo-micrograph - X 550 diameters. Vesicles are large and filled with Colloid. The lining epithelial cells are Cubical. The Colloid is stained Orange and appears dark grey in the Section. Aniline blue - Orange G. preparation.

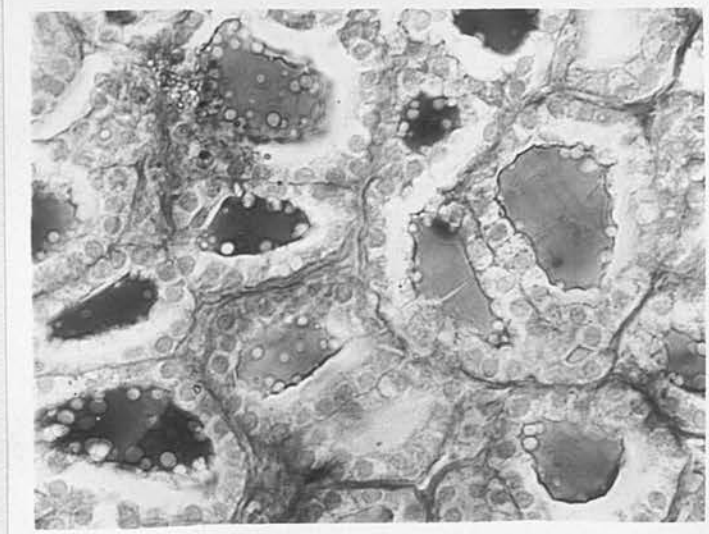

FIG: 19. Thyroid of an anaemic female Cat fed with Ventriculin. Photo-micrograph. X 550 diameters. Vesicles are small. The black colloid is stained blue and the grey colloid Orange. Colloid shrunken. Lining epithelial cells mostly cubical. Aniline blue - Orange G. preparation.

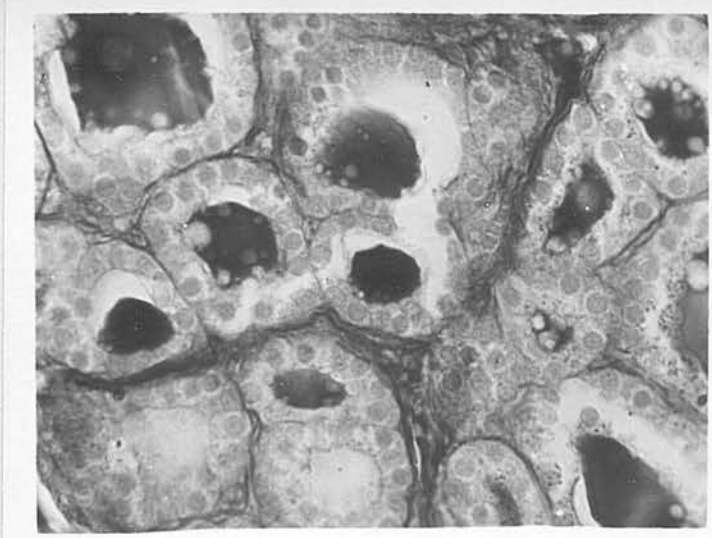

FIG: 20. Thyroid of an anaemic female Cat fed with liver. Photo-micrograph. X 550 diameters. Most of the Vesicles are small, Containing darkly stained Colloid which does not Completely fill them. The lining epithelial cells are almost Columnar. Aniline blue - Orange G. preparation.

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