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Simple Experimental Anemia and Liver Extracts.

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In 1920 Whipple, Robscheit and Hooper[1] reported that liver feeding had a very favorable influence on the production of red cells and hemoglobin in dogs during short anemia periods. Since that time Whipple and Robscheit-Robbins have shown that in long standing severe anemia in dogs a liver diet has an extraordinary effect. In fact a diet of liver (beef, pig, sheep, calf or chicken) will produce a maximum regeneration of blood hemoglobin and red cells under standard experimental conditions.[2]

During this considerable experimental period we have tested from time to time a variety of liver extractives. Dr. Hooper in San Francisco tested an alcohol-ether extract upon a few cases of severe pernicious anemia with results which at that time were believed to be negative. In our work we have paid particular attention to changes in the hemoglobin index during these diet periods. We have used a considerable variety of food materials and extracts in an effort to modify the hemoglobin index. The hemoglobin index is obtained by dividing the hemoglobin expressed in per cent by the red cell hematocrit expressed in per cent. At times it appeared that we could decrease the hemoglobin index by certain foods or extracts (Table I) which might indicate a more rapid formation of stroma than of hemoglobin so that the cells would be less completely saturated with hemoglobin. Again we noted an increase in the hemoglobin index which might indicate a more rapid production of hemoglobin than of stroma. These changes are not great and are inconstant, but we believe these changes are significant and in time will be fully understood.

Changes in the hemoglobin index are of particular interest in relation to the recent important work of Minot and Murphy,[3] who give evidence that liver diet is important in the clinical treatment of pernicious anemia. From the standpoint of the pathologist we have pointed out[4] the observed facts in pernicious anemia which in our judgment indicate a *faulty blood construction* rather than an *increased blood destruction* as the essential feature in this obscure disease. The experiments of Cohn, Minot, Fulton, Ulrichs, Sargent, Weare and Murphy[5] tend to support this view and the evidence may

finally show that in pernicious anemia we are dealing with a real *deficiency disease* in which the stroma construction material is wanting.

Because of the work of Minot and his colleagues dealing with the influence of certain liver extractives upon human cases of clinical pernicious anemia, we felt it would be of interest to report briefly some of our work on liver extracts as applied to simple, long-continued secondary anemia in dogs. It will be seen from the tables below that all the liver fractions contain some potent substances which are capable of influencing somewhat the regeneration of hemoglobin and red cells under standard conditions. The sum of these reactions does not equal the favorable reaction of whole liver feeding, so that one may assume considerable loss in the method of extract preparation.

Method.

The general experimental method has been described elsewhere.[6] The liver fractions were prepared as follows: To 1000 grams of fresh ground beef liver were added 3000 cc. of 1/10 per cent HCl—the mixture brought to a boil and continued boiling for 2 minutes. The clear filtrate was concentrated to about 1/4 the original volume

TABLE I.

Water extract of beef liver.

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REGEN'D BLED
Food, grams per day	%	*kgm.*	*cc.*	*mil.*			%	%	*gm.*
Dog 24-26 Bull, male, adult.									
Fresh apples 200, bread 300	88	10.4	592	4,6	0.42	2.01	19.4	39	1.1
Fresh apples 200, bread 300, salm. 75	85	10.0	606	4,8	0.45	2.05	20.9	43	1.3
Water liver ext. 500 cc. (eq. 500 g. fr. liver),	100	10.6	594	5,7	0.45	2.06	21.2	44	13.1
bread 400, salm. 50	100	10.7	594	5,2	0.48	1.90	21.5	41	11.6
Bread 400, salm. 50	98	10.6	580	5,4	0.43	1.99	23.3	46	1.3
Dog 24-49 Bull, female, adult.									
Bread 400, salm. 75	100	14.2	786	5,8	0.37	1.83	23.7	43	1.3
Bread 400, salm. 75	96	14.7	828	5,5	0.45	2.11	19.9	42	15.3
Water liver ext. 500 cc., bread 400	100	14.5	806	5,5	0.43	2.01	23.5	47	1.4
Water liver ext. 500 cc., bread 400	100	14.2	808	6,6	0.42	1.81	22.1	40	20.4
Bread 400	100	14.2	808	5,8	0.39	1.89	24.3	46	1.3

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}$$

over steam. This represents the water extract given in Table I—fed readily by mixing with the dry standard bread.

The coagulated liver brei remaining was then extracted with 2 volumes of 95 per cent grain alcohol at 38° C. for 24 hours—a second extraction was done in similar fashion and these two alcoholic extracts combined and evaporated over steam to a thick paste. This fraction represents the alcoholic extract used in Table II.

The residue of liver after water and alcohol extraction as described above was freed from alcohol by boiling with water. This is the liver residue fed in Table III.

Table I shows that this watery extract of liver contains some materials favorable for hemoglobin and red cell regeneration in standard anemia conditions. Dog 24-26 ate all of the food mixture and shows an increased production per 2 week period of about 20 grams of hemoglobin above the base line level of control periods. Dog 24-49 shows about the same increased production of 20 grams above control periods. The increase of 15 grams hemoglobin in the control fore-period is due to a "carry over" from the preceding favorable diet period not shown in the table (diet of dried apricots). We observe a *fall in the hemoglobin index* in both experiments which may indicate that stroma production may be in advance of hemoglobin production. These dogs received a watery extract, the equivalent of 500 grams fresh liver daily, added to a ration of standard bread 400 grams daily. Dog 24-26 received in addition 50 grams canned salmon daily as an appetizer.

TABLE II.
Alcoholic extract of beef liver.

DIET PERIODS 1 WEEK EACH.	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. RE-M'V'D BLED
Food, grams per day	%	*kgm.*	*cc.*	*mil.*			%	%	*gm.*
Dog 24-22 Coach, female, adult.									
Bread 375, salm. 50	100	11.9	700	5,5	0.40	1.94	22.7	44	1.3
Alcoh. liver ext. 26, br. 400	100	12.0	648	5,5	0.43	1.85	25.4	47	1.3
(eq. to 500 g. fresh liver)	100	12.0	664	6,0	0.42	1.86	29.4	55	14.2
Bread 400, salm. 50	97	12.0	653	5,2	0.48	1.86	22.9	43	12.0
Bread 400, salm. 50	96	12.1	730	4,7	0.49	2.03	22.7	46	1.2
Dog 18-114 Bull, female, adult.									
Alcoh. liver ext. 26, br. 350	94	15.0	779	4,4	0.53	2.04	22.8	46	1.5
(eq. 500 g. fr. liver), salm. 75	90	15.2	819	4,9	0.53	2.08	22.0	46	17.7
Bread 375, salm. 75	67	14.5	714	4,8	0.47	2.00	22.5	45	1.4

Table II shows that the alcoholic extract likewise contains some material favorable for hemoglobin and red cell regeneration. Dog 24-22 ate all of the material and shows an increased production per 2 week period of about 22 grams of hemoglobin above the control periods. Dog 18-114 under the same condition shows a little less production of hemoglobin—about 16 grams per 2 week period. This dog did not eat quite all the material. These dogs received an alcoholic extract the equivalent of 500 grams fresh liver daily added to a ration of standard bread.

TABLE III.

Beef liver residue after water and alcoholic extractions.

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. RE-M'V'D BLED
Food, grams per day	%	*kgm.*	*cc.*	*mil.*			%	%	*gm.*
Dog 24-25 Bull, male, adult.									
Bread 400	100	13.0	733	5,7	0.43	1.96	25.2	49	1.5
Liver residue 275, bread 300	87	13.7	815	6,2	0.43	1.86	26.8	50	14.8
(eq. to 500 g. fresh liver)	100	13.8	717	6,4	0.47	1.90	26.8	51	18.7
Bread 400	100	13.8	778	5,0	0.49	2.08	23.5	49	1.3
Bread 400	100	13.6	778	5,7	0.48	1.92	28.6	55	13.1
Dog 19-104 Bull, male, adult.									
Bread 275, salm. 75	84	11.3	634	4,8	0.50	2.11	22.7	48	1.4
Bread 275, salm. 75	84	11.3	652	4,5	0.49	2.06	21.2	44	1.2
Liver residue 275, bread 225	77	11.7	720	4,1	0.52	1.89	22.7	43	1.1
Liver residue 150, bread 200	55	11.2	622	6,3	0.48	1.97	22.1	44	17.1
Bread 250, salm. 75	47	10.3	615	5,2	0.42	1.95	22.5	44	1.3

Table 3 shows that the liver residue after water and alcoholic extraction still contains substances favorable for hemoglobin and red cell regeneration in standard anemia conditions. Dog 24-24 ate practically all of this residue and shows an increased production per 2 week period of about 35 grams of hemoglobin above the control level. This includes a "carry over" of about 12 grams in the second week after-period due to the reserve stored during the favorable diet period of liver residue feeding. Dog 19-104 ate about two-thirds of the liver residue, was given less during the second week, and partly on that account, produced less hemoglobin per 2 week period, about 15 grams.

From these few experiments one may not draw conclusions, but the following points may be made with some security. All three

of these fractions prepared from fresh liver contain substances which are favorable for hemoglobin and red cell regeneration in simple anemia in dogs. In round numbers we may say the watery fraction produces per 2 week period in excess of about 20 grams—the alcoholic fraction perhaps a little less than 20 grams of hemoglobin and the liver residue perhaps 20 to 30 grams of hemoglobin per 2 week period above control levels. The sum of the three extracts would be about 60 to 70 grams hemoglobin per 2 week period. This corresponds to 80 to 100 grams hemoglobin per 2 week period which would be about the average value of the fresh liver fed to the same standard anemia dogs.

[1] Whipple, G. H., Robscheit-Robbins, F. S., and Hooper, *Am. J. Physiol.*, 1920, liii, 236.

[2] Robscheit-Robbins, F. S., and Whipple, G. H., *Am. J. Physiol.*, 1925, lxxii, 408.

[3] Minot and Murphy, *J. Am. Med. Assn.*, 1926, lxxxvii, 470.

[4] Whipple, G. H., *Arch. Int. Med.*, 1922, xxix, 728.

[5] Cohn, Minot, Fulton, Ulrichs, Sargent, Weare and Murphy. Paper read before the Federation of American Societies for Experimental Biology, Rochester, N. Y., April, 1927.

[6] Whipple, G. H., and Robscheit-Robbins, F. S., *Am. J. Physiol.*, 1925, lxxii, 395.

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Effect of Extracts of Pars Tuberalis of Hypophysis on Urine Secretion.

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Saline suspensions or boiled acidulated extracts of the bovine *pars tuberalis* when injected intravenously into rabbits under paraldehyde anesthesia produce a diuresis differing from that produced by injection of posterior lobe extracts. The resulting diuresis comes on without a marked rise of blood pressure and without the latent period of 3 to 5 minutes (during which the flow of urine is entirely suppressed) which are characteristic accompaniments of posterior lobe diuresis.

Diabetes insipidus is held[1, 2] to be due to injury of the base of the brain in the region of the *tuber cinereum*. Critical examination of the figures of Bailey and Bremer,[2] and of Curtis,[3] shows that in practically every instance the injury has involved the *pars tuberalis* of the hypophysis.