6234

Action of Human "Pernicious Anemia Liver Extract."

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How the antianemic principle in liver which is active in pernicious anemia produces its effect is not known. Neither is it known whether the active principle is stored and depleted, like certain vitamins, nor if the liver *in vivo* secretes it in a hormone-like manner for the purpose of governing hematopoiesis. It was considered desirable to ascertain whether the liver of a pernicious anemia patient contains the antianemic principle. Since an opportunity to perform 2 experiments bearing on this question occurs so rarely at present, we desire to record the results we have obtained. The livers of 2 pernicious anemia patients were extracted. The first extract was made from a liver of a pernicious anemia patient who had received liver extract prior to her death sufficient to produce a reticulocytosis, the bone marrow at autopsy showing an active hemopoietic response. The liver was extracted and the extract administered intravenously to 3 pernicious anemia patients with positive results. The second extract was made from the liver of a case of pernicious anemia who entered the hospital in a moribund condition and died shortly after entrance. Because of her rapid exitus, the liver extract she received intramuscularly and intravenously did not produce an active hemopoiesis, which was shown by the absence of reticulocytosis and hemopoietic activity in the bone marrow. An extract was made from this liver and administered to a pernicious anemia patient subcutaneously with no result.

Experiment I. A white female patient, 75 years old, entered the hospital in a semi-stuporous state. Pernicious anemia had been diagnosed 5 years previously. The classical findings with achlorhydria and spinal cord complications were present. The patient had received therapy in a haphazard manner during the past 5 years. During the past month she had been confined to bed but had received no therapy for a period of months. In addition to the presence of pernicious anemia, evidences of cystitis, nephritis, and myocarditis were present at this time. The urine was 3 plus for albumin and contained many leucocytes. The blood urea was 45.5;

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creatinine, 2.8; blood sugar, 111; red blood count, 750,000; hemoglobin, 27; white blood cells, 3,600; a blood film characteristic of pernicious anemia. The patient had a septic temperature and her condition became progressively worse. Liver extract for parenteral injection not being available, the patient was started on liver extract by mouth on September 19th, and was given 1600 cc. of normal saline and 1000 cc. of glucose solution hypodermically. The patient was given an average of 3½ oz. of potent liver extract in small divided dosages daily, receiving a total of 42 oz. during the 12 days of the treatment, each ounce equivalent to 240 gm. of whole liver. Because of her poor condition, she was also given 400 cc. of whole blood intravenously. The day of the transfusion, or on the seventh day of liver therapy, the blood examination revealed R.B.C., 1.67; Hb.,41; W.B.C., 9,100; reticulocytes, 25.4%. The patient rallied for several days and appeared to be improved but died 7 days after the transfusion from the complications. An immediate autopsy showed generalized severe anemia (pernicious type); combined degeneration of spinal cord; pseudo-membranous cystitis; septic tumor of spleen; parenchymatous degeneration of liver, kidneys, and myocardium; severe coronary sclerosis; bone marrow showed evidence of hyperactive erythropoiesis with similar embryonic cells in splenic meshes.

The liver was immediately extracted by a slightly modified method for preparing fraction G (Minot) for intravenous use. Eight hundred grams of liver were available for extraction. Animal tests for toxicity of the prepared extract showed it to be non-toxic for dogs. Thirty cc. of the final extract was equivalent to 100 gm. of liver.

Three patients with pernicious anemia were given the extract intravenously to determine whether the active "anti-pernicious anemia" principle is present in the liver of the pernicious anemia patient. The 3 patients receiving the extract responded. After the response was obtained, the patients were placed on liver extract (Chapell, Rockford, Illinois) by mouth, a typical liver therapy remission resulting.

Case 1. Typical case of pernicious anemia. A young woman with first relapse of six weeks' duration. Results recorded in Table I show a marked reticulocyte rise following 4 daily injections of human pernicious anemia liver extract, a total of 14 cc. intravenously.

Case 2. An old case of pernicious anemia with several previous relapses, who failed to react to liver by mouth. A woman 68 years of age. Results in table show a reticulocyte response following

TABLE I.
Response of 3 Pernicious Anemia Patients to Intravenous Administration of Extract of Liver of a Pernicious Anemia Patient.
Case on Liver Therapy Sufficient to Cause Reticulocytosis.

Patient No.		Days 1	2	3	4	5	6	7	8	9	10	11	12
1	Dose cc.	3.5	3.5	3.5	3.5								
	Hb %	17				20					23		
	RBC	0.89				0.83					1.39		
	WBC	3.6				2.8					4.0		
	Reticulocytes %	0.2	1.4	1.4	1.6	2.4	3.8	7.4	16.4	27.4	32.6		
2*	Dose cc.		3.0										
	Hb. %	29			41					44			
	RBC	0.89			1.08					1.49			
	WBC	4.7			4.3					3.6			
	Reticulocytes %	1.4			0	8.4	5.0	4.2		0.4			
3	Dose cc.		4.0	3.5	3.5		4.0		3.5				
	Hb. %	53					57						69
	RBC	1.8					1.86						2.74
	WBC	6.0					5.4						5.6
	Reticulocytes %	0	0	0	0	0.8	2.8	2.6	1.8	1.4	2.4	2.4	0.4

* Failed to react to liver extract by mouth.

only one intravenous injection (3 cc.) of human pernicious anemia liver extract.

Case 3. Pernicious anemia with a somewhat higher initial Hb. and R.B.C. count in male, 63 years of age, associated with a chronic myocarditis. Results recorded in table show no marked reticulocyte response but a marked increase in Hb. and R.B.C. in 11 days following intravenous injections of human pernicious anemia liver extract. Also followed by a marked clinical improvement. Patient received 5 intravenous injections, a total of 18½ cc.

The results, which are definitely positive, show that the active antianemic principle in pernicious anemia is present in the liver of the pernicious anemia patient recently treated with liver extract by mouth. The fact that one patient gave a response with 3.0 cc. of the extract (the equivalent of 10 gm. of liver) and the fact that Case 1 responded very well with 14 cc., leads us to believe that the active principle is present in approximately the same concentrations as in normal cattle liver.

Experiment II. A white female patient, 55 years of age, entered the hospital in a semi-stuporous state. The history obtained was that she had been ailing for the past 4 years. Tachycardia and paroxysmal attacks of dyspnea began 4 years ago following an illness which at that time was diagnosed as heart disease. Gastrointestinal disturbances were also experienced since then, consisting of occasional attacks of anorexia, and diarrhea. She was fairly comfortable and able to carry on her daily activities until 7 months

ago, when she began feeling weak. The weakness became progressively worse so that in the past month she was confined to bed. Anorexia and vomiting occurred more frequently in the past months, associated with loss of appetite, strength and weight. She had never been on any form of liver or liver extract therapy throughout her entire illness.

The essential findings on entrance were an icteric tinge of the skin and mucous membranes, an atrophic tongue, a slightly enlarged heart, with a rapid, regular apex beat of 160 per minute, and a slightly enlarged liver and spleen. The blood and spinal Wassermanns were negative. The urine was negative. The blood urea was 32.69; creatinine, 2.05; blood sugar, 136; red blood count, 810,000; hemoglobin (Sahli), 29; white blood cells, 3,900; and a blood film characteristic of a primary anemia of the pernicious type. A suitable donor for a blood transfusion was unobtainable. She was started on parenteral liver extract, receiving a total of 7 cc. of which 4 cc. was given intramuscularly and 3 cc. intravenously. Her general condition became worse, the patient expiring 5 days after her entrance to the hospital. Daily reticulocyte counts were made, but no response was recorded. Autopsy showed severe generalized anemia (pernicious type); fatty changes and hemosiderosis of the liver; severe fatty degeneration of the myocardium; arterio- and arteriolosclerosis of the kidneys; atrophy of the gastric mucosa; chronic recurrent verrucous endocarditis of the mitral valve with insufficiency, hyperplasia of the bone marrow, but no hyperactive response as observed in the previous case.

The liver was extracted as in the previous case. From 2 to 4 cc. was injected daily for 9 days subcutaneously into a typical case of pernicious anemia. The reticulocyte count was taken daily and a complete count at 5 day intervals. The patient received a total of 31 cc. of liver extract (100 gm.) subcutaneously. Daily reticulocyte counts were taken, but no response was recorded in 13 days. The patient was then given one ounce of Chapell's liver extract t.i.d. by mouth which was followed by the usual hemopoietic response (Table II).

The results in this experiment show that the liver of this pernicious anemia patient who died before she had received effective liver therapy, did not contain the antianemic principle active in pernicious anemia.

The results of these 2 experiments indicate that the "specific" antianemic substance in liver is absent from the liver of the patient suffering from pernicious anemia and that when the antianemic

TABLE II.

Response of Typical Case of Pernicious Anemia Patient to Extract of Liver of a Pernicious Anemia Patient Who Received an Insufficient Amount of Liver Therapy to Produce Hemopoietic Response Because of Her Sudden Exitus.

Patient No.		Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
4	Dose cc.	2	4	0	4	4	4	4	4	5	*	*	*	*	*	*	*
	Hb. %	41					34			30					35		
	RBC	1.63					1.24			1.04					1.23		
	WBC	2.5					3.0			3.6					5.0		
	Reticulocytes %	0	0	0.8	0.6	1	0	0	0.2	0.4	0.4	0.4	0.2	1	3.4	13.9	27.8

* Failed to respond to this human liver extract so was started on 10th day with potent liver extract by mouth, one ounce t.i.d. showing typical reticulocyte response.

substance is administered to the pernicious anemia patient the liver stores it. This observation denotes that the active principle is similar in certain respects to the vitamins, particularly the "fat soluble" vitamins in regard to storage and depletion. Clear cut experimental evidence shows that Vitamins A, D, and E are stored and rather rapidly depleted, and also that B and G are stored and depleted, the evidence not being so clear cut in the case of the latter 2. Vitamin C is stored and depleted in the guinea pig, but in the rat either the rate of utilization of C is very slow, or it is synthesized or produced during digestion. However, the C content of skeletal muscle appar-

ently disappears before a detectable change occurs in the liver. The fact that the antianemic principle in liver for pernicious anemia is present in other tissues, but in less concentration, is analogous to the distribution of vitamins, also that a definite latent period precedes the development of anemia when liver therapy is discontinued, is analogous to the latent period characteristic of vitamin deficiency.

On the basis of the observations of Castle,[1] which indicate that gastric digestion in normal man releases substances from meat that have an antianemic value in pernicious anemia, the most logical interpretation of our results is that the "active principle" of liver is not a fixed or integral part of liver protoplasm and may be stored and released physiologically by the liver *in vivo* to govern hematopoiesis.

6235

A New Disease of Moose. II.

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(Introduced by F. W. Tanner.)

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Thomas and Cahn[1] described a new disease of moose *(Alces americana americana)* in northern Minnesota. The symptoms of the disease were described, with the associated blood picture. Transplanting ticks *(Dermacentor albipictis)* from an infected moose to guinea pigs and rabbits produced a disease showing identical symptoms, with the same associated blood picture as in the moose. Profound cellular changes in the blood elements and the presence of bacteria were mentioned. The organism was isolated. The cellular changes will be discussed in the final report; the present paper deals with the isolated organism.

The first recognized bacteria-like organism was found in smears of the intestinal contents of ticks engorged with the blood of diseased moose. With a modified Wright stain these organisms showed a capsulated coccoid bacterium. This intestinal content was cultured on dextrose agar, and the organism isolated. A saline suspension of this pure culture was inoculated intravenously into

[1] Castle, *Am. J. Med. Sci.*, 1929, **178**, 748, 764.

[1] Thomas, L. J., and Cahn, A. R., *J. Parasit.*, 1932, **18**, 4.