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Amount of Material Effective in Pernicious Anemia Present in Dog Liver.

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Addisonian pernicious anemia may be considered to be a dietary deficiency disease usually conditioned by the absence of a specific "intrinsic factor" from the gastric secretion.[1] It is believed that in the normal person or animal the interaction of this gastric factor which is thermolabile, and a dietary factor associated in many substances with the vitamin B complex, results in the storage of a thermostable substance in the liver. The presence of the thermolabile factor has been demonstrated in human[1, 2] and bovine[3] gastric juice and in the stomach tissues of the hog.[4] Thermostable material has been found in the livers of these 3 species,[5, 6] as well as in those of sheep,[6] deer,[6] horses,[7] and fish.[8] Although present in greatest concentration in the liver, the thermostable material is also found in kidney,[6] brain,[9] placenta[10] and other organs. The demonstration of the presence of either the thermolabile or the thermostable material depends upon the typical response of suitable patients with pernicious anemia to the administration of the organs or their extracts referred to above. In the case of the thermolabile factor an opportunity for contact with the dietary factor under appropriate conditions is essential to the test.[1]

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[1] Castle, W. B., and Townsend, W. C., *Am. J. Med. Sc.*, 1929, **178**, 764.

[2] Wilkinson, J. F., *Brit. Med. J.*, 1930, **1**, 236. Barnett, C. W., *Am. J. Med. Sc.*, 1931, **182**, 170. Singer, K., *Klin. Wchnschr.*, 1932, **11**, 1459.

[3] Williams, H. A., and Vanderveer, J. B., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 858.

[4] Sharp, E. A., *J. Am. Med. Assn.*, 1929, **93**, 749. Sturgis, C. C., and Isaacs, R., *J. Am. Med. Assn.*, 1929, **93**, 747.

[5] Unpublished Observations.

[6] Minot, G. R., Murphy, W. P., and Stetson, R. P., *Am. J. Med. Sc.*, 1928, **175**, 578.

[7] Richter, O., Meyer, A. E., and Ivy, A. C., *J. Am. Med. Assn.*, 1932, **98**, 1623.

[8] Connery, J. E., *Am. J. Med. Sc.*, 1930, **180**, 603. Davidson, L. S. P., *Brit. Med. J.*, 1932, **2**, 347.

[9] Ungley, C. C., *Lancet*, 1931, **2**, 63.

[10] Mach, R. S., *Sang*, 1931, **5**, 299.

Gutzeit and Herrmann[11] have reported finding the specific intrinsic factor in the gastric juice of the dog. On the contrary, Ivy and his associates[12] have failed to obtain any effect in Addisonian pernicious anemia by the administration of desiccated dog stomach and have also failed to produce pernicious anemia in the dog by total gastrectomy.[13] If the dog's gastric juice contains the specific intrinsic factor, it is reasonable to assume that "liver extract", potent in Addisonian pernicious anemia, ought to be stored in the livers of normal dogs If, on the contrary, the dog's gastric juice does not contain the intrinsic factor, as is suggested by the observations of Ivy, the dog's liver should not contain the thermostable principle. In order to determine the "liver extract" content of canine liver, 4 identical extracts suitable for intramuscular injection were prepared from normal dog livers. For control purposes 4 similar extracts were prepared from normal hog livers. The method of preparation of each of these extracts was as follows: The original quantity of liver pulp, as well as the volumes of filtrates and concentrates at each stage of the process, was kept as nearly as possible the same in each preparation. Fresh liver was finely ground, and the pulp separated from the fibrous tissue in a separatory grinder. Canine extract No. 1 was made from a single dog liver. Portions of the livers of 10 different dogs were used in the preparation of canine extract No. 2, and of 4 and 3 different dogs in the preparation of canine extracts No. 3 and No. 4, respectively. The pulp was added to twice its weight of tap water, the mixture stirred vigorously and allowed to stand over night in a refrigerator. The mixture, after being heated rapidly to the boiling point, was acidified to pH 6, and filtered through hard paper by suction, following which the residue was re-extracted with half the original quantity of water and again filtered. Sufficient 95% alcohol was added to the combined filtrates to make a final concentration of 70% alcohol. The filtrate, after standing over night, was then separated from the precipitate and evaporated *in vacuo* at a temperature of less than 45°C. to a small volume. Sufficient 95% alcohol was then added to make the concentration 70%, the filtrate was again separated from the precipitate and sufficient absolute alcohol added to make the final concentration 95% alcohol. The precipitate, after sedimenting over night, was washed with ether, dried and dissolved in water so that 10 cc. of the solution was derived from 100 gm. of fresh liver pulp. The

[11] Gutzeit, K., and Herrmann, J., *München. med. Wchnschr.*, 1931, **78**, 266.

[12] Ivy, A. C., Richter, O., and Kim, M. S., *Proc. Am. Physiol. Soc., Am. J. Physiol.*, 1932, **101**, 59.

[13] Ivy, A. C., Morgan, J. E., and Farrell, J. I., *Surg. Gynec. Obst.*, 1931, **53**, 611.

TABLE I.

Daily Administration by Intramuscular Injection of Liver Extracts Derived from the Amounts of Fresh Liver Indicated.

Days	First Periods. Canine Extract.							
	No. 1, 20 gm.		No. 2, 100 gm.		No. 3, 100 gm.		No. 4, 100 gm.	
	Case No. 1		Case No. 2		Case No. 3		Case No. 4	
	R.B.C. mils.	Retics. %	R.B.C. mils.	Retics. %	R.B.C. mils.	Retics. %	R.B.C. mils.	Retics. %
0	1.86	1.8	2.05	0.6	1.68	1.8	1.51	0.6
2	1.78	2.2	2.08	0.8	1.52	2.2	1.37	0.6
4	1.73	0.6	2.11	0.8	1.85	5.0	1.51	1.2
6	1.74	0.1	2.18	2.0	1.92	**8.8**	1.55	7.2
8	1.46	1.0	2.20	2.2	2.00	6.2	1.51	17.8
10	1.39	1.2	2.17	**4.4**	2.33	4.6	1.74	**20.6**
			Second Periods. Hog Extract.					
	No. 1, 20 gm.		No. 2, 25 gm.		No. 3, 100 gm.		No. 4, 100 gm.	
2	1.38	2.2	2.40	3.6	2.31	1.2	1.92	15.6
4	1.44	2.8	2.32	2.0	2.27	0.8	1.96	15.0
6	1.51	6.6	2.34	1.8	2.41	1.8	2.24	11.6
8	1.92	10.7	2.23	1.4	2.85	1.0	2.38	3.8
10	1.98	**13.5**	2.58	1.0	2.80	1.2	2.36	4.8
12	2.05	7.8	2.68	1.2	2.72	0.8	2.75	2.6

reaction was adjusted to pH 7.4, 0.3% tricresol added and the material passed through a Berkefeld filter, following which it was stored in sterile vials kept in a refrigerator.

The results of the intramuscular administration of these extracts are shown in Table I. To each of 4 patients canine liver extract was administered for 10 days and then immediately followed during the next 10 days by hog liver extract. No demonstrable effect, either upon the reticulocytes or upon the red blood corpuscles, was obtained when the extract from 20 gm. of canine liver was injected daily in Case 1. Subsequently, in the same patient, a satisfactory response occurred following the administration of a similar extract derived from the same quantity of hog liver. The second patient, Case 2, showed a small reticulocyte response and a moderate gain of erythrocytes following the daily injection of extract derived from 100 gm. of dog liver. During the second period, this patient was given hog liver extract daily in amounts derived from 25 gm. of liver. No secondary reticulocyte response occurred. The third patient, Case 3, showed a moderate response of reticulocytes and a gain of over half a million red blood cells in a period of 10 days, during which daily injections of the extract derived from 100 gm. of dog liver were administered. A similar amount of hog liver extract was administered to this patient during the subsequent period with no second reticulocyte response. The fourth patient, Case 4, showed a maximum reticulocyte response during the daily

intramuscular injection of the extract derived from 100 gm. of dog liver, without a secondary increase of reticulocytes during the administration of similar amounts of hog liver extract.

These observations demonstrate that the liver of the dog contains thermostable material effective in pernicious anemia, although in lesser quantities than does hog liver. As far as we have been able to ascertain, practically all other mammalian livers thus far assayed, have contained the same relative concentration of effective material. The hog, ox, sheep, horse and deer, however, are all essentially herbivorous animals, whereas the dog belongs to the carnivora. A partial deficiency of either the dietary or the gastric factors could theoretically account for the diminished quantity of potent substance in the liver of the dog. Judging from their state of nutrition, the diet of these animals appeared to be satisfactory and, therefore, presumably contained adequate amounts of the factor associated with the vitamin B complex. On the other hand, the contradictory observations[11, 12] cited above, upon the content of intrinsic factor of the dog's stomach, suggest an explanation for the decreased potency of the dog liver. It is possible that a decreased amount of intrinsic factor is present in the gastric juice of the dog and that this results in a decreased amount of thermostable effective material in the liver.

Conclusions. 1. Extracts made from normal dog livers produce on intramuscular injection typical remissions in patients with Addisonian pernicious anemia. 2. The content of potent material in canine liver appears to be only about one-fifth that of hog liver. 3. This fact may possibly be correlated with the contradictory findings concerning the amount of the specific intrinsic factor in canine gastric juice.

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Treatment of Pellagra by Means of Parenteral Liver Extract.

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Goldberger and Sebrell[1] found that liver extract fed to dogs in large amounts either prevented or retarded the development of

[1] Goldberger, J., and Sebrell, W. H., *J. Am. Med. Assn.*, 1932, **99**, 95.