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The Possible Relationship Between Absorption of B. Welchii Toxin and Pernicious Anemia.*

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The experiments reported here are in continuation of our investigation of the possible relationship between the absorption of *B. welchii* toxin from the intestine, and the condition of pernicious anemia. In a previous communication[1] there was described a severe type of anemia produced in monkeys by the intravenous inoculation of small doses of potent *B. welchii* toxin. In all the animals, however, after 3 weeks treatment, an immunity became apparent which was not broken down even by a greatly increased dosage, although abnormalities of erythrocytes and a leucopenia persisted for some time.

It was next attempted by the continued inoculation of dosages of *B. welchii* toxin just below the limit of toleration, to cause such serious injury to the hematopoietic system as might prove irreparable, and to induce a progressive degenerative condition resembling that of pernicious anemia. The method of toxin preparation was essentially as described heretofore, except that somewhat larger pieces of fresh sterile pigeon muscle were added to the casein digest broth. Employing our pernicious anemia *B. welchii* strain, "Navitol", sterile toxins were obtained which were lethal for pigeons within 24 hours after intramuscular inoculation of 0.5 cc. Two young ring-tail monkeys *(Cebus capucinus)* were given a long series of intravenous toxin inoculations in dosage sufficient to keep the animals in a condition of more or less severe anemia for 9 to 10 weeks, in contrast with the 3 or 4 weeks period of the previous experiment. After about 10 weeks, however, in spite of greatly increased dosage, it was not possible to check the tendency of reversion toward normal, and we became convinced that intravenous inoculations of *B. welchii* toxin would not damage the hematopoietic tissues to such a degree that the condition was irreversible.

It was then decided to determine the effect following the introduction of the toxin through the more or less inflamed or injured gastro-intestinal mucous membrane. For, if the absorption of *B. welchii*

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TABLE I.
Effect on the Blood of Monkeys of Prolonged Intravenous Inoculations of *B. welchii* Toxin.

Dates	Erythrocyte Count		Hemoglobin Per Cent		Color Index		Anisocytosis		Poikilocytosis		Nucleated Red Cells*		Punctated Basophiles		Leucocyte Count	
1926	J	K	J	K	J	K	J	K	J	K	J	K	J	K	J	K
1/13	4,600,000	4,780,000	86	92	0.93	0.9	—	—	—	—	—	—	—	—	6,800	6,600
1/15	2,790,000	1,960,000	76	54	1.4	1.4	++	—	—	—	—	—	—	—	10,800	8,800
1/18	4,500,000	1,290,000	85	23	0.94	0.9	+++	+++	—	—	2	3	—	—	6,200	4,200
1/19	2,920,000	1,510,000	60	24	1.4	0.8	++++	—	+	—	1	1	—	—	10,000	4,200
1/21	2,060,000	1,170,000	50	24	1.25	1.0	++++	++++	+++	++	6	—	+	—	11,000	4,600
1/25	2,500,000	2,510,000	68	55	1.36	1.1	++	—	—	+++	2	—	—	—	4,000	4,400
1/26	1,700,000	2,070,000	50	49	1.47	1.2	++++	+++	+	+	22	3	++	—	11,200	8,000
1/28	1,740,000	1,530,000	55	32	1.6	1.0	++++	++++	++	—	3	10	—	—	9,600	7,800
2/3	2,080,000	1,630,000	46	50	1.15	1.59	+	—	+	—	—	—	—	—	4,400	4,400
2/8	2,060,000	2,410,000	63	59	1.57	1.2	++	+++	++	++	—	—	—	—	4,600	6,800
2/18	2,040,000	2,090,000	63	65	1.57	1.6	—	++	—	—	—	—	—	—	6,800	6,600
2/26	2,480,000	2,090,000	66	45	1.5	1.1	—	+	—	—	3	—	—	—	4,600	4,800
3/3	2,530,000	1,940,000	65	58	1.3	1.5	—	+++	+++	++	3	—	—	—	8,600	5,600
3/10	2,510,000	1,990,000	68	67	1.4	1.8	—	—	—	—	—	—	—	—	6,600	7,200
3/19	3,120,000	2,640,000	80	78	1.27	1.5	++	+	++	+++	—	—	—	—	5,600	6,200
4/3	4,090,000	3,190,000	87	61	1.08	0.9	—	—	—	—	—	—	—	—	5,900	4,800

Inoculations and examinations about every three days: Jan. 14-30, 0.5 to 2.5 cc.; Feb. 1-Mar. 1, 3 to 10 cc.; Mar. 1-15, 10 to 20 cc.
*Number per 100 leucocytes counted.

toxin is the factor of primary importance in the etiology of pernicious anemia, it probably gains entrance to the vascular system from this locality.[2] Experiments conducted some time previously had indicated that this toxin introduced into normal intestines of monkeys was not absorbed therefrom, at least not to a degree sufficient to cause pronounced changes in the blood.

About this time the work of Leake and Ritchie[3] came to our attention. They found that the repeated treatment of the stomach of atropinized dogs with 5 per cent sodium fluoride would give rise in time to a condition of atrophic gastritis, with a subsequent complete achlorhydria and pronounced anemia. Their methods, kindly supplied by Dr. Leake, were applied to monkeys in a modified form, with the purpose of increasing the absorption of *B. welchii* toxin from the gastro-intestinal tract. At weekly intervals 3 monkeys: M, O and P, ½ hour after an intramuscular injection of ⅓ mg. of atropin, were fed 8 cc. of 1 to a 3 per cent aqueous solution of sodium fluoride through a stomach tube. A 5 per cent strength, tried at first, proved fatal to the animal. The fluoride was allowed to remain in contact for 7 minutes, during which time the animal was rolled about to insure contact with all surfaces of the stomach. Then 15 cc. of a 1 per cent aqueous solution of calcium carbonate was introduced through the tube, to precipitate the fluoride and finally, as much as possible of the stomach content was aspirated. Complete emptying generally resulted. In addition, 2 days following the fluoride treatment, monkeys M and O were fed weekly by stomach tube, 20 cc. of a whole fluid culture of *B. welchii* (Navitol strain) prepared as described heretofore. Monkey P served as a control for the fluoride alone.

It was found that 3 per cent sodium fluoride was about the maximal strength which could be used safely, and after one or two treatments the dosage was reduced to 1 per cent. The fluoride caused an acute catarrhal inflammation of the gastric mucosa, which tended to subside in 24 to 48 hours. The pathological changes following long continued treatment are described later and the details of the treatment and its effects on the blood are reported in Table II.

As indicated, the 2 monkeys treated with the fluoride, and also the toxin died, 1 of them (O) after 60 days and the other, (M) after 103 days, both of them showing an extreme degree of anemia. The control (P), however, was alive and in good condition after 115 days when it was chloroformed, 39 days after the fluoride treatments had been stopped. The control showed a loss of weight of 8.7 per cent (996 to 875 grams) as contrasted 23.3 per cent (1356 to 1040 grams) for monkey M. About 8 weeks after the start of

TABLE II.

Effect of Absorption of *B. welchii* Toxin from the Gastro-intestinal Tract on the Blood of Monkeys.

M and O, Toxin treated. P, Control.

Dates of blood exam.	Erythrocyte Count in Millions			Hemoglobin Per Cent			Color Index			Leucocyte Count in Thousands			Anisocytosis			Poikilocytosis			Nucleated Red Cells per 100 leucocytes		
	M	O	P	M	O	P	M	O	P	M	O	P	M	O	P	M	O	P	M	O	P
Normal 4/1	4.61	4.94	4.99	89	92	96	0.96	0.93	0.97	10.0	8.6	7.4	—	—	—	—	—	—	0	0	0
4/20	2.36	2.36	2.96	68	67	71	1.5	1.5	1.2	11.0	9.0	11.2	++	++	+	--	--	--	0	0	0
4/26	2.75	2.68	3.90	63	70	74	1.1	1.3	0.94	5.2	9.6	9.0	++	+++	+	--	--	--	0	0	0
5/3	2.93	2.78	3.76	67	69	78	1.1	1.2	1.0	9.6	10.0	9.2	+	+	--	--	--	--	0	0	0
5/10	2.39	2.73	3.96	65	64	76	1.4	1.2	0.97	8.8	8.0	10.8	+	+	--	+	--	--	1	0	0
5/17	2.00	1.65	3.09	55	55	67	1.3	1.7	1.0	5.8	5.6	8.2	+	++	--	+	++	--	0	1	0
5/24	1.61	1.86	4.07	53	50	75	1.6	1.3	0.93	7.2	8.2	9.2	+	++	+	--	--	--	0	0	0
6/2	3.17	2.73	3.52	70	55	62	1.0	1.0	0.88	8.0	10.0	7.6	++	+++	+	++	--	--	0	0	0
6/9	2.45	1.97	3.05	59	53	72	1.2	1.3	1.2	5.6	8.8	8.2	+	+++	--	--	++	--	0	0	0
6/16	1.51	Died 6/10	2.43	33		65	1.1		1.3	4.6	—	8.0	+		++	---		--	6	0	0
6/23	1.29	—	3.00	35		70	1.0		1.2	3.2	—	7.8	+++		+	++		--	1		0
6/30	0.91	—	2.78	10		62	0.55		1.1	3.2	—	6.2	++++		--	+		--	4		0
	Died 7/2	—	—																		
7/8	—	—	2.95			62			1.0			6.4			+			--			0
7/14	—	—	2.04			70			1.7			8.8			+-			--			0
7/21	—	—	3.16			65			1.0			6.2			+-			--			0

Sodium fluoride treatments:
M, total 9, March 31 to June 12, 1926.
O, total 8, April 6 to June 9, 1926.
P, total 11, April 5 to June 14, 1926.

B. welchii toxin treatments:
M, total 13, April 1 to June 24, 1926.
O, total 8, April 20 to June 4, 1926.
P, control, no *B. welchii* toxin.

the treatments, the reaction of the gastric contents 3 hours after a meal was taken. All 3 monkeys showed a H-ion reading of about 5.0, as contrasted with 4.6 for 2 untreated monkeys on the same diet. Thus at this state, at least, the fluoride had not caused any notable decrease in gastric acidity.

The blood examination data in Table II presents very definite evidence for the absorption of *B. welchii* toxin. This is indicated not alone by the lower erythrocyte count, the Hb per cent and higher color index for M and O than for P, but also in the much more marked degree of anisocytosis, and at times, of poikilocytosis in the 2 former animals at nearly every examination. Particularly striking are the differences revealed at the May 24th examination, when the blood condition of the control P was nearly normal, whereas M and O both showed severe anemia.

It is of interest, too, that an immunity did not develop when the toxin was administered under these conditions. Monkey O died about 8 hours after a treatment with 3 per cent sodium fluoride, but M died 20 days after the fluoride, but not the toxin treatments, had been stopped. This finding may be contrasted with the immunity regularly developing during intravenous inoculations. Whether or not this difference was due solely to the fluoride factor has not as yet been determined.

Monkey M, about 2 weeks before death, passed into a profound state of anemia, showing an erythrocyte count of 1,510,000 hemoglobin 33, and a marked leucopenia.

At necropsy all the organs of M showed an extreme degree of anemia but no marked lesions. The spleen and retroperitoneal lymph nodes were not enlarged. The bones were dead white and brittle, and the marrows softer and lighter red than is normal for these monkeys. On the other hand, the organs of P were only moderately anemic, and the bone marrows were a much darker red than in the case of M.

The microscopical findings, as reported by Dr. James Ewing, proved of particular interest. The stomach of the control P monkey revealed evidence of a mild catarrhal gastritis with considerable lymphocyte infiltration of the mucosa, but the glands and acid cells stained well and appeared normal. In contrast the stomach of M exhibited a very severe catarrhal gastritis, desquamation of the acid cells, and well marked lymphocyte infiltrations of the submucosa. No glands with intact secreting cells were noted. The greater interest, however, pertains to the microscopical differences in the marrows. Comparing the femur marrows of the 3 monkeys the findings

were as follows: That of control P was normal in appearance, being solidly packed with normal marrow cells in normal relations. That of O appeared very cellular and congested, mostly large and small lymphocytes, islands of nomoblasts missing but a good many nucleated hemoglobin-holding cells which are much larger than usual; no evidence of blood destruction. The marrow of M, the monkey which had received 5 more toxin feedings than O, appeared very cellular. The rather numerous fat cells are undergoing mucinous degeneration and their envelopes are often broken. There is a moderate deposit of recent blood pigment in the macrophages. No islands of nucleated reds are seen, but there are many scattered nucleated reds, staining poorly. The impression is gained of a severe toxic degeneration of the marrow tissue affecting fat cells and erythrocytes, both with and without nuclei. Unfortunately for the purposes of this comparison the control P was not killed until 5½ weeks following the last sodium fluoride treatment. The blood condition, however, had remained much the same and it is not believed that the marrow findings would have been materially different at an earlier examination.

These tissue findings, as well as those for the blood, present definite evidence of the absorption of *B. welchii* toxin through the sodium fluoride injured gastro-intestinal mucosa, with resulting pathological changes in the blood and tissues markedly over and above that caused by fluoride alone. The failure of these animals to develop a resistance to the toxin, as has invariably followed intravenous inoculation, may have been due to the depressing influence of the chemical, or possibly to the portal entry of the toxin. Further study is being given of this mode of treatment on antitoxin production. This is a complete report.

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[1] Kahn, M. C., and Torrey, J. C., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 8.

[2] Moench, L. M., Kahn, M. C., and Torrey, J. C., *J. Infect. Dis.*, 1925, xxxvii, 161.

[3] Leake, C. D., and Ritchie, G., *Am. J. Physiol.*, 1926, lxxvi, 234.