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## VITAMIN B<sub>12</sub> IN THE BLOOD AND IN THE LIVER OF NORMAL AND OF TOTALLY GASTRECTOMIZED PIGS\*

By

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PETRI, S., C. PETRI and FOLKE RASMUSSEN: *Vitamin B<sub>12</sub> in the blood and in the liver of normal and of totally gastrectomized pigs.* Acta vet. scand. 1973, 14, 421—427. — The concentration of vitamin B<sub>12</sub> in the blood was studied in five totally gastrectomized pigs followed for 10—18 months, and in four controls followed for 5½—8 months. The concentration of vitamin B<sub>12</sub> in the liver was investigated in three pigs of each group. The content of vitamin B<sub>12</sub> in the blood was of the same magnitude in both groups. Thus, removal of the stomach does not compromise the normal presence of this vitamin in the blood. The B<sub>12</sub> content in the liver was also unaffected by the operation, whereas it was increased after simultaneous administration of vitamin B<sub>6</sub>.

vitamin B<sub>12</sub>; gastrectomy; pig; blood; liver.

The object of the present study was to determine directly the content of vitamin B<sub>12</sub> in the blood and in the liver of totally gastrectomized pigs. The absorption of this vitamin in pigs submitted to the operation has previously been investigated in fewer cases by *Heinrich* (1957), and by *Prinz et al.* (1959) who used the Schilling test.

### MATERIAL AND METHODS

The experiment comprises nine female pigs of the Danish land race, weighing from 12 to 19 kg at the outset. Five were

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totally gastrectomized (Nos. 4, 10, 11, 14, 15) and followed for 10—18 months. Four were controls (Nos. 5, 6, 12, 13) followed for 5¼—8 months. The iron-deficiency nature of the gastroprival anaemia was concurrently studied in two of the pigs (Nos. 11, 14) by *Petri et al.* (1970). Supplementary to previous studies on the role of vitamin B<sub>6</sub> in gastrectomized pigs (*Petri et al.* 1944, *Petri & Petri* 1970) pig No. 15 was treated with pyridoxine hydrochloride, 0.3—1 mg/kg twice weekly i.m. throughout the experimental period.

The standard fodder mixture, without addition of vitamins B, consisted of 68.3 % barley, 12 % oats, 16 % soya meal, 2 % meat and bone meal, 0.13 % vitamin supplement (4 i.u. A, 1 i.u. D, and 20 µg E per g of fodder mixture), and 1.57 % mineral mixture. The pigs were allowed fodder in accordance with their body weight. Water ad libitum. Before surgery atropine sulphate (0.05 mg/kg) was injected i.m. Anaesthesia was induced by i.m. injection of 2 mg/kg phencyclidine (Sernylan®) and maintained by 1.5—2.5 % halothane (Fluothane®) in oxygen administered by mask as described by *Gyrd-Hansen* (1971).

Total gastrectomy was performed in the usual way, establishing end-to-end anastomosis of the oesophagus and duodenum according to *Petri* (1955).

During the first 4 days after the operation the pigs were given 16,000 i.u. benzylpenicillin and 20 mg dihydrostreptomycin per kg body weight (Streptipen-prokain Rosco vet.®). The vitamin B<sub>12</sub> content in the blood serum during the experimental period (Fig. 1) was determined by the method of *Hoff-Jørgensen* (1954) and *Kristensen* (1958). Immediately after sacrifice, the content of vitamin B<sub>12</sub> in the samples of liver tissue was determined by the same method. The determination of B<sub>12</sub> in the blood serum was performed by E. Hoff-Jørgensen in the Department of Biochemistry, Royal Dental College, Copenhagen, and that of B<sub>12</sub> in liver tissues by Tage Hansen, Dumex Ltd., Copenhagen.

## RESULTS

Throughout the experimental period the vitamin B<sub>12</sub> level in the blood serum was the same and constant in all the pigs, the four control ones as well as the five gastrectomized ones (Fig. 1).

The average blood level of B<sub>12</sub> in the controls and in the gastrectomized pigs was 103.2 picog/ml ± 32.7 and 90.0 picog/ml

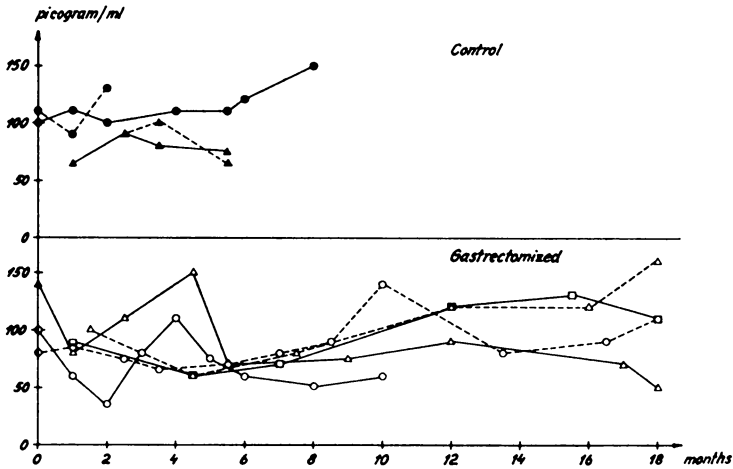


Figure 1.

**Control Pigs**

Vitamin B<sub>12</sub> in the blood serum from four pigs (Nos. 5 (●—●), 6 (●--●), 12 (▲—▲), and 13 (▲--▲)).

**Totally Gastrectomized Pigs**

Vitamin B<sub>12</sub> in the blood serum from five pigs (Nos. 4 (○—○), 10 (○--○), 11 (△—△), 14 (△--△), and 15 (□—□)). No significant difference from the control pigs.

± 32.7, respectively. Statistical analysis of the results showed no significant difference ( $P > 0.005$ ) in the blood level of vitamin B<sub>12</sub> between the two groups.

The vitamin B<sub>12</sub> content in the liver tissue from three of the controls and two of the gastrectomized pigs was in the same range (Table 1) and  $10^3$ — $10^4$  of that in the blood serum. On the other hand, the B<sub>12</sub> content in the liver of the gastrectomized pig treated with vitamin B<sub>6</sub> (No. 15) was greater than in the other two operated pigs.

Table 1. Concentration of B<sub>12</sub>-vitamin in liver tissue.

Control pigs, Nos.	Concentration of B <sub>12</sub> µg per		Totally gastrectomized pigs, Nos.	Concentration of B <sub>12</sub> µg per	
	kg tissue	g nitrogen		kg tissue	g nitrogen
5	216	10.5	10	685	17.7
12	519	15.0	14	384	12.8
13	281	11.9	15	944	32.2

## DISCUSSION

The absorption of vitamin B<sub>12</sub> from the gastrointestinal tract is generally considered to depend predominantly upon the intrinsic factor (IF).

The finding of normal levels of this vitamin in the blood must be considered to be based upon this factor — in the totally gastrectomized pigs as well as in the controls.

Since the pyloric area — the IF area in the pig stomach — has been eliminated by the gastrectomy, the formation of the absorption-conditioning complex is likely to have taken place in the duodenum. The presence of IF in this site in the pig has been pointed out by, amongst others, *Sharp et al.* (1931), *Meulengracht* (1935), *Geiger et al.* (1940/41), *Landboe-Christensen & Bohn* (1947), *Landboe-Christensen & Hoff-Jørgensen* (1955), and *Hoedemaeker* (1965). *Uotila* (1938) also ascribed an IF function to the ileum, whereas *Håkansson et al.* (1971), amongst others, ascribed it to a specific paracrine cellular system in the gastrointestinal tract.

Theoretically the conditions for an optimal binding between IF and vitamin B<sub>12</sub> in the intestinal canal might have changed postoperatively. According to *Heinrich* (1957) the absorption of vitamin B<sub>12</sub> in physiological conditions takes place without the mediation of IF.

Thereby, any microbial synthesis of vitamin B<sub>12</sub> in the intestinal tract would possibly manifest itself (*Harper* 1961).

The liver of the operated pigs cannot have exercised a compensatory maintenance of the normal blood level of the vitamin. The vitamin B<sub>12</sub> content in the liver tissue of the experimental pigs was in the same range as in eight other pigs aged 3—12 weeks. In the former this cannot have been due to the presence of a depot of the vitamin at the commencement of the experiment, when the total liver weight made up about  $\frac{1}{10}$  of its weight at the conclusion of the experiment.

The terminal normal values of vitamin B<sub>12</sub> in the liver of the gastrectomized pigs followed for many months militate against a release of this vitamin from the liver to the blood. The vitamin B<sub>12</sub> content of normal pigs rapidly subsides when it is lacking in the diet (*Johnson* 1957, *Wolff* 1957).

In contradistinction to our results *Heinrich* as well as *Prinz et al.* (1959) found an increased absorption of vitamin B<sub>12</sub> in

totally gastrectomized pigs. In explanation, they presumed that apart from IF the stomach contained absorption-inhibiting properties (Glass 1963, pp. 606 and 719—724). The gastrectomy was supposed to have eliminated these properties and thus to have made possible an increased absorption of the vitamin.

A perceptible disagreement with respect to pigs has also been found between previous studies on the causal significance of gastrectomy in the complete disappearance of the antipernicious anaemic liver principle (Bence 1933, Geiger *et al.*, Petri *et al.* 1941) and the present finding of a normal quantity of vitamin B<sub>12</sub> in the liver following the same operation. This might be due to hitherto unelucidated differences in the diet (Ivy 1940). Dedichen (1946) reported that a liver extract from "winter-fed" Norwegian pigs was not, or practically not, active against pernicious anaemia.

In one of the gastrectomized pigs (No. 15) given pyridoxine hydrochloride (vitamin B<sub>6</sub>) parenterally a high content of vitamin B<sub>12</sub> in the liver was seen. In earlier experiments vitamin B<sub>6</sub> given parenterally had prevented or partly cured the symptoms from the central nervous system constantly seen in totally gastrectomized pigs (Petri *et al.* 1944; Petri & Petri 1970). The possible relation between these two observations needs further experimental data.

The progressive, very marked subsidence of vitamin B<sub>12</sub> in the blood and in the liver following total gastrectomy in man cannot be compared causally with our experiments on pigs. In man the operation removes the sole localization of IF (in the fundic glandular area). In the pig the IF of the pyloric area is removed, it is true, but it is left in the duodenum and possibly in the small intestine.

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## SAMMENDRAG

*Vitamin B<sub>12</sub> i blod og lever fra normale og total gastrektomerede grise.*

Koncentrationen af vitamin B<sub>12</sub> blev bestemt i blodet fra fem total gastrektomerede grise over en periode på 10—18 måneder, samt i fire kontrolgrise, som blev fulgt i 5¼—8 måneder. Koncentrationen af vitamin B<sub>12</sub> i levervævet blev bestemt hos tre grise fra hver gruppe. Indholdet af vitamin B<sub>12</sub> i blodet var af samme størrelsesorden hos de to grupper af grise. Indholdet af vitamin B<sub>12</sub> i leveren ændredes ikke efter gastrektomien, hvorimod det øgedes ved indgift af vitamin B<sub>6</sub>.

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