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ANEMIA AND GROWTH RETARDATION IN TOTALLY GASTRECTOMIZED SWINE*

By

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PETRI, SVEND, FOLKE RASMUSSEN and CLAUS PETRI: *Anemia and growth retardation in totally gastrectomized swine*. Acta vet. scand. 1980, 21, 197—208. — The effects of total gastrectomy in six young swine were followed and described during an experimental period up to 18 months. Two of the gastrectomized pigs (Nos. 1 and 2) were medicated with cycobemin every second week. Two other gastrectomized pigs (Nos. 11 and 14) were medicated with iron-dextran twice a week. Three non-gastrectomized swine receiving the same feed as the gastrectomized animals were observed as controls for up to eight months.

One of the gastrectomized non-medicated pigs (No. 4) did not gain weight, while the other gastrectomized animals had a normal appearance and gained weight but less than the controls. The non-medicated as well as the cycobemin-medicated gastrectomized animals developed a microcytic hypochrome anemia corresponding to the anemia in iron deficiency. Histochemically loss of iron in the depots (liver, spleen, bone marrow and intestine) was demonstrated in the gastrectomized animals except those treated with iron-dextran. Histological and histochemical examinations of the nervous system did not show any abnormalities.

g a s t r e c t o m y ; s w i n e ; a n e m i a .

Total gastrectomy in swine has been done for various purposes a.o. for use as models for the experimental pernicious anemia in humans (*Petri et al.* 1937, 1970, *Petri* 1955).

The object of the present study was to continue this research work, to describe the biological effect of the feed used now (*Petri et al.* 1973) and to discuss these findings in relation to those found in earlier experiments in swine given another type of feed (*Petri et al.* 1937, *Petri*).

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MATERIALS AND METHODS

Thirteen female swine, Danish landrace, weighing from 12 to 19 kg at the start of the observations were used. Three were controls (Nos. 5, 12, 13) and observed for five to eight months. Ten (Nos. 1, 2, 3, 4, 7, 8, 9, 10, 11, 14) were totally gastrectomized (Petri et al. 1973) at the onset of the experiment. Four (Nos. 3, 7, 8, 9) died few days after the surgery, while the others were observed for 10—18 months. The surgery and post-operative treatment have been described previously (Petri et al. 1973). The feed, without addition of any B vitamins, 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) and 1.6 % mineral supplement (including 0.1 mg cobalt sulfate per g of supplement).

The effect of intramuscular administration of iron-dextran (Imferon, 50 mg Fe⁺⁺⁺/ml, 1 ml i.m. twice weekly to swine Nos. 11 and 14) or vitamin B₁₂ (inj. cycobemini 30 µg/ml, 2 ml i.m. every second week to swine Nos. 1 and 2) was studied from the start of the experiments.

The vitamin B₁₂ content in the blood serum during the experimental period was determined by the method of Hoff-Jørgensen (1954) and Kristensen (1958).

Routine standard methods were used for the estimation of hemoglobin, erythrocytes, volume of erythrocytes (hematocrit), average erythrocyte volume, leucocytes, serum iron (transferrin-bound iron), protein, urea, creatinine, sodium, potassium, calcium, chloride and phosphate each month throughout the first six months of the experimental periods and then every two to three months. The swine were killed and examined post mortem. Fifty tissue samples taken from various parts of the intestinal tract, glands, liver, kidney, spleen, bone marrow, and central as well as peripheral nervous system were fixated in phosphate-buffered 3.6 % formaldehyde solution (pH 7.0), dehydrated and imbedded in paraffin. Sections from these organs, cut 6 µm thick, were used for routine histology: hematoxylin and eosin, van Gieson Hansen stainings.

Histochemically carbohydrates and conjugated proteins were demonstrated in the lysosomes of epithelial cells and connective tissue cells (McManus 1946, 1948). Iron pigment was identified in the cytoplasm of the reticulo-endothelial cells and in the sur-

face epithelium of the gastrointestinal tract (*Perls 1867, Bunting 1949*). A semiquantitative evaluation of the results of the histochemical reactions for iron was performed.

On sections from the nervous system, myelin (*Weil 1933*), nerve fibers and nerve endings (*Bodian 1936*) were shown. Histochemically nucleic acids were demonstrated in the Nissl's substance and in the nuclei of nerve cells and glial cells (*Einarson 1951*). Lipids were localized to the lysosomes of nerve cells, glial cells and in the myelin sheaths (*Lison 1934, Lillie & Ashburn 1943, Adams 1959*).

RESULTS

Performance

The six totally gastrectomized swine except one (No. 4) had a clinically normal appearance, i.e. normal uptake of feed (in fractionated portions four times a day), and skin, hair, carriage, movements and feces were normal. Coprophagi was not observed. These five totally gastrectomized swine gained weight throughout the experimental period but less than the normal controls (Fig. 1). The lengths (cm) were normal in relation to the body

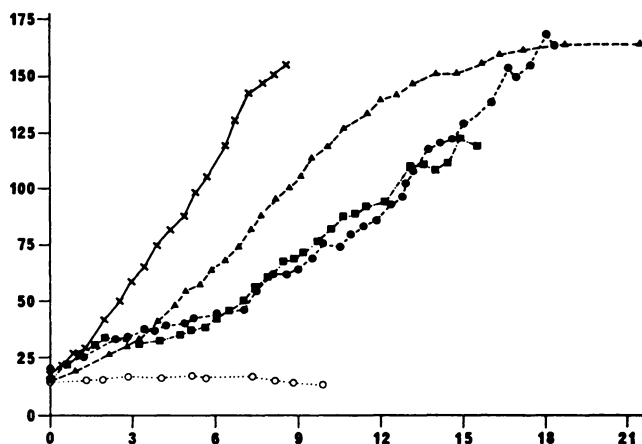


Figure 1. Body weight in relation to age. Three control pigs (×—×), two totally gastrectomized (No. 4 ○---○, No. 10 ●---●), two totally gastrectomized treated with iron-dextran (▲----▲) and two totally gastrectomized treated with cycobemin (■-----■). Ordinate: Body weight in kg. Abscissa: Months after start of experiment.

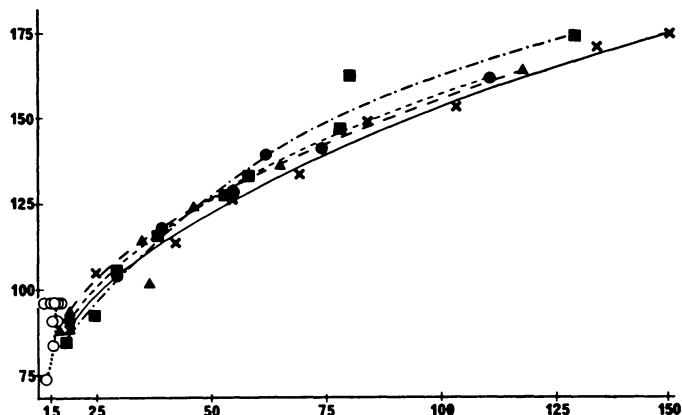


Figure 2. Body weight in relation to length. Symbols as in Fig. 1. Ordinate: Length in cm. Abscissa: Body weight in kg.

weight (Fig. 2). From Figs. 1 and 2 it is seen that the retardation of growth is pronounced in pig No. 4. In all animals the feed uptake was normal according to body weight, but feces usually more dry than normal. The skin was seborrheic and brownish. The hair longer than normal and woolly especially in pig No. 4. Atrophy of the skeletal muscles was pronounced in pig No. 4. The movements were a little stiff, but no symptoms were observed from the central nor the peripheral nervous system.

Blood and serum parameters

Most parameters measured during the experimental period in gastrectomized swine were similar to those seen in control animals (Table 1). Some parameters, however, demonstrated differences between control and gastrectomized animals. These differences are illustrated in Figs. 3—7.

Serum-iron measurements showed that the concentration of transferrin-bound iron was similar in control animals and in two totally gastrectomized swine injected iron-dextran intramuscularly. The content of serum iron in the other four totally gastrectomized animals decreased rapidly to about 1/3 of the level in the controls (Fig. 3).

Erythrocyte volume, i.e. hematocrit, and blood hemoglobin values are demonstrated in Figs. 4 and 5 and indicate constant and identical values in the control as well as in

Table 1. Blood and serum parameters. Average values of 6—14 observations in each pig during the experimental period.

Pig No.	Number of erythrocytes $\times 10^{12}/l$ blood	Number of leucocytes $\times 10^6/l$ blood	Protein g/l serum	Urea g/l serum	Creatinine mg/l serum	Sodium mmol/l serum	Potassium mmol/l serum	Calcium mmol/l serum	Chloride mmol/l serum	Phosphate mmol/l serum
Control										
5	7.33	15.7	65	0.28	15	141	3.8	2.48	99	2.89
12	6.96	18.5	68	0.23	12	144	3.9	2.30	99	2.47
13	8.65	20.0	65	0.24	15	139	3.7	2.45	99	2.89
Gastrectomized										
4	7.82	13.0	65	0.28	12	132	4.2	2.31	92	2.62
10	7.92	11.4	68	0.37	17	137	4.3	2.28	96	2.94
1 ^a	7.06	12.6	66	0.33	15	137	3.9	2.23	98	2.70
2 ^a	8.38	11.7	70	0.35	16	138	4.4	2.31	97	3.09
11 ^b	6.36	15.4	68	0.27	14	142	3.8	2.47	98	2.69
14 ^b	6.17	14.6	71	0.28	16	141	3.9	2.61	96	2.88

^a Treated with vitamin B₁₂, 60 µg cycobemin every second week.

^b Treated with iron-dextran, 50 mg Fe⁺⁺⁺ twice a week.

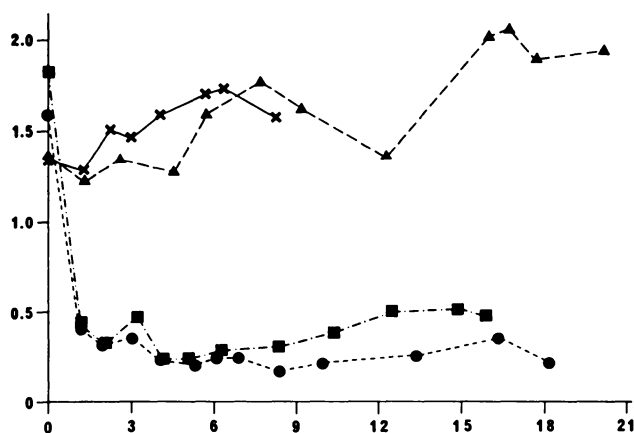


Figure 3. Serum iron (transferrin-bound) during the experimental period. Three control pigs (x—x), two totally gastrectomized (●—●), two totally gastrectomized treated with iron-dextran (▲—▲) and two totally gastrectomized treated with cycobemin (■—■).

Ordinate: Concentration of iron (transferrin-bound), mg/l.

Abscissa: Months after start of experiment.

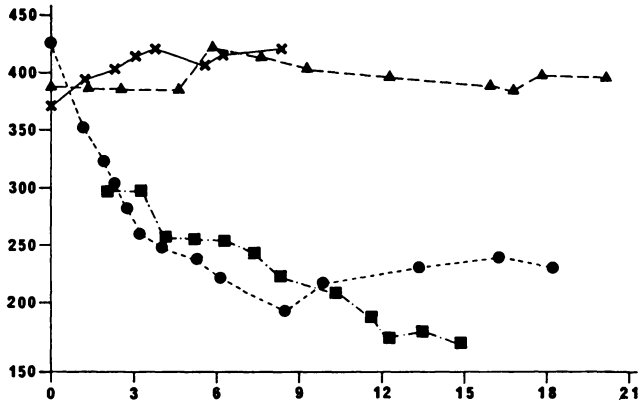


Figure 4. Erythrocyte volume (hematocrit) during the experimental period. Symbols as in Fig. 3.
 Ordinate: Erythrocyte volume, ml/l.
 Abscissa: Months after start of experiment.

the iron-treated totally gastrectomized animals. In the totally gastrectomized animals without and with parenteral application of B₁₂ the erythrocyte volume and hemoglobin were steadily decreased to about 50 % in nine months (Figs. 4 and 5).

Mean erythrocyte volumes (MCV) are shown in Fig. 6. Again the values in the controls and in the iron-treated

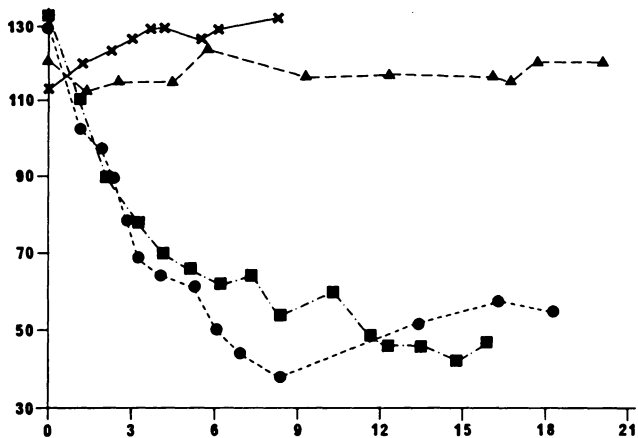


Figure 5. Hemoglobin in blood during the experimental period. Symbols as in Fig. 3.
 Ordinate: Hemoglobin in g/l.
 Abscissa: Months after start of experiment.

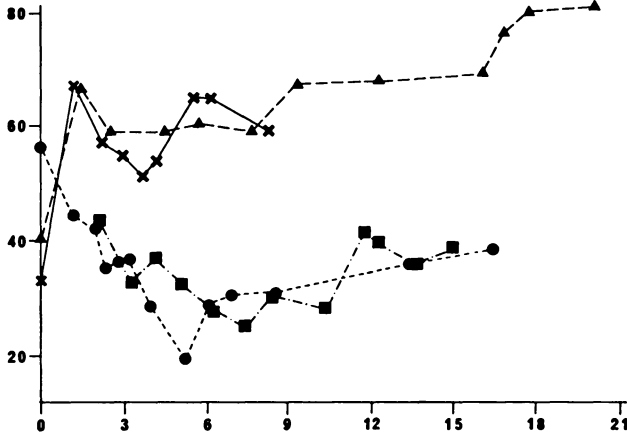


Figure 6. Mean erythrocyte volume (MCV) during the experimental period. Symbols as in Fig. 3.
 Ordinate: Mean erythrocyte volume, fl.
 Abscissa: Months after start of experiment.

totally gastrectomized animals are identical, while those in non-treated as well as B₁₂-treated totally gastrectomized animals were decreased to 50 % during the first five to seven months.

Cycohem in, i.e. vitamin B₁₂, was estimated in serum throughout the experiment and results are given in Fig. 7. The concentration of cycohem in serum was constant and of the

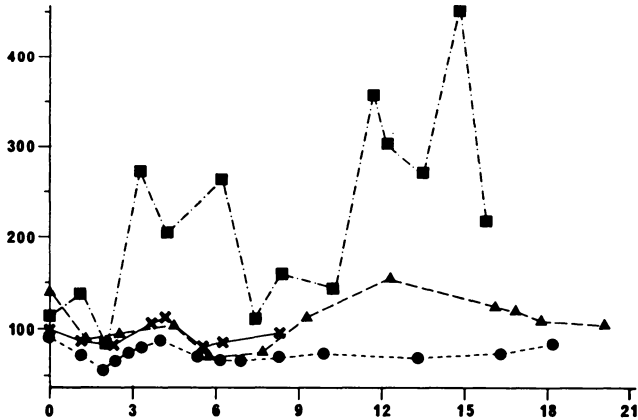


Figure 7. Vitamin B₁₂ in blood serum during the experimental period. Symbols as in Fig. 3.
 Ordinate: Vitamin B₁₂ in blood serum, ng/l.
 Abscissa: Months after start of experiment.

same order in controls, totally gastrectomized animals, and totally gastrectomized given iron-dextran parenterally. The totally gastrectomized animals injected cycobemin intramuscularly had a higher but not a constant concentration of cycobemin in serum (Fig. 7).

Histology

Histology showed a pronounced hyperplasia of the bone marrow and a histochemically verified loss of iron in the depots (liver, spleen, bone marrow and intestine) in the non-iron-treated totally gastrectomized animals. Bone-marrow was normoplastic and the iron depots were packed in the gastrectomized swine parenterally injected iron. The results of the histochemical reaction for iron are listed in Table 2. In the iron-depleted swine the blood smears showed microcytosis, hypochromia, aniso- and poikilocytosis. The histological and histochemical appearances of the nervous system were without abnormalities. All the other

Table 2. Results of histochemical reaction for the demonstration of iron (Perls' reaction).

		Controls without gastrectomy and without iron me- dication (Nos. 5, 12, 13)	Gastrectomized animals with post- operative iron medication (i.m.) (Nos. 11, 14)	Gastrectomized animals without post-operative iron medication (i.m.) (Nos. 1, 2, 4, 10)
Duodenum	SE	+	o	o
	LP	++	o	o
Jejunum	SE	o	o	c
	LP	+	+	c
Ileum	SE	o	o	c
	LP	o	++	c
Colon	SE	o	o	c
	LP	+	++	o
Liver		++	+++	o
Spleen		++	+++	o
Bone marrow		++	+++	o

SE = surface epithelium.

LP = lamina propria.

o no reaction.

+ weak reaction.

++ moderat reaction.

+++ strong reaction.

organs were normal, with the exception of a severe simple diffuse atrophy in the growth-retarded swine (No. 4). In none of the gastrectomized animals a compensatory hyperplasia of the stomach glands was observed.

DISCUSSION

The smaller daily weight gain in totally gastrectomized swine was expected and had also been observed earlier (*Petri et al.* 1937, *Petri* 1955, *Cunha* 1957). A total stop in growth — as in swine No. 4 — is also described earlier (*Petri et al.* 1937). From the present experiments it is impossible to explain this observation and the difference between swine No. 4 and No. 10 (Fig. 1).

The lowered values of iron in serum, hemoglobin and mean erythrocyte volume in gastrectomized animals not treated with iron-dextran and the severe hyperplasia of the bone marrow indicate development of a microcytic, hypochrome anemia with total depletion of iron depots in spite of the fact that all animals had received feed sufficient in iron. The hyperplasia of bone marrow, and the microcytic, hypochrome anemia with depletion of iron depots are verified by histological and histochemical examinations (*Harper* 1961).

The totally gastrectomized swine (Nos. 11 and 14) getting iron-dextran parenterally did not develop the above-mentioned specific hematopoietic changes including decrease in serum iron. *Maison & Ivy* (1933/34) demonstrated a certain effect of parenteral application of Fe_3NH_4 -citrate in swine but the erythrocyte volume was lower than normal. *Cunningham* (1967) prevented anemia in totally gastrectomized swine by injections of vitamin B_{12} (400 μg) and iron-dextran (500 mg) once a week. The present results showed that parenteral application of cycobemin alone failed to prevent anemia in totally gastrectomized swine (Nos. 1 and 2).

The results obtained in the present experiments showing anemia and arrest of growth are in full agreement with earlier observations (*Petri et al.* 1937, 1940). The totally gastrectomized swine in these experiments did not develop clinically or histologically observable changes in central or peripheral nervous systems as reported earlier (*Petri et al.* 1937, 1940, *Nørgaard* 1942). A possible explanation for this discrepancy is the difference between the standard feed with additives used in these experi-

ments and the feed (barley, oats, potatoes, ryebread, skim milk) used in earlier experiments. An obvious difference between these two types of feed is that the content of cobalt in the feed used in these experiments is much higher than in that used earlier (0.44 mg Co/kg v. 0.02 mg/kg). A high content of cobalt in fodder increases the microbiological formation of vitamin B₁₂ in sheep (Hedrich et al. 1973) and in golden hamsters (Tseng et al. 1976).

CONCLUSION

Total gastrectomy in swine eliminates a factor important for the absorption of iron from the intestinal tract. Parenteral treatment with cycobemin failed to prevent anemia, while parenteral application of iron-dextran prevented a development of anemia. Total gastrectomy did not change the content of vitamin B₁₂ in blood serum. Clinically and histologically no changes in the nervous system were observed.

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SAMMENDRAG

Anæmi og hæmmet tilvækst hos totalt gastrektomerede svin.

Virksomheden af total gastrektomi af 6 pattegrise blev undersøgt og beskrevet over en forsøgsperiode på op til 18 måneder. To af de gastrektomerede svin (nr. 1 og 2) blev behandlet med cycobemin hver anden uge og 2 andre (nr. 11 og 14) blev behandlet med jern-dextran 2 gange pr. uge. Tre ikke-gastrektomerede svin fungerede som kontrol-dyr og fik samme foder som de gastrektomerede svin. Kontrol-dyrene blev observeret i op til 8 måneder.

Et af de gastrektomerede, ubehandlede svin (nr. 4) tog ikke på i vægt. De øvrige gastrektomerede svin var normale af udseende og tog på i vægt, men tilvæksten var mindre end hos kontrol-dyrene. Både de cycobemin-behandlede og de ubehandlede gastrektomerede svin udviklede en mikrocytær hypokrom anæmi svarende til en jernmangel anæmi. Ved histokemisk undersøgelse påvistes tab af jern fra depoterne (lever, milt, knoglemarv og tarm) hos alle gastrektomerede dyr, som ikke var blevet behandlet med jern-dextran. Ved de gennemførte histologiske og histokemiske undersøgelser af nervesystemet kunne der ikke påvises forandringer i vævene.

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