HISTOPATHOLOGICAL STUDIES ON SO-CALLED KUWAZU DISEASE (COBALT DEFICIENCY) IN CATTLE IN JAPAN

I. FINDINGS OF MAIN ORGANS AND TISSUES

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(Received for Publication March 10, 1963)

INTRODUCTION

Since ruminants were brought to South and Western Australia by the early settlers, the term "Coast Disease"³¹⁻³⁴ or "Denmark wasting disease"¹⁰, both of which were used by the inhabitants, has long been well-known to Australian farmers. These diseases are enzootic nutritional ones with the same etiology. MARSTON³²⁻³⁴ and LINES³¹ carried out experimental studies on these diseases. UNDERWOOD and FILMER⁵⁰ studied them independently in South and Western Australia in 1935. It was demonstrated that small supplements of soluble cobalt salts were completely effective to prevent or treat them. FILMER¹⁰ was the first to report the histopathology of these diseases in 1933. He gave these diseases the collective name "enzootic marasmus" and pointed out that they were similar to "bush sickness" in New Zealand, "nakuritis" in Kenya, and "salt sickness" in Florida.

Affected cattle^{9,10,34} reveal early clinical symptoms several months after introduced in enzootic areas, where they cannot grow nor thrive. Occasional deaths are encountered among grazing flocks and herds continuously depastured on certain restricted areas in the countries mentioned above. The main symptoms of the disease are anorexia, allotriophagy, progressive emaciation, listlessness, and lack of co-ordination in the movement due to general anemia. Sometimes it is difficult to find any specific symptoms of the disease.

It was demonstrated that cobalt supplements administered *per os* had a dramatic response. The etiology of the similar diseases^{9,29,36,51} remained unknown for a long time at the end of last century, but was found to respond to cobalt. There are, however, some other diseases of ruminants in different areas which have similar symptoms. They are "Morton-Main disease" in New Zealand¹, "pine" in Scotland^{5,8,41}, "Grand Traverse or shore disease" in northern Michigan², some ailment in the Hebrides Islands, Canada⁷, and Wisconsin²³, and "neck ailment" in Massachusetts⁴.

In several districts^{10-20,23,26,27,33-35,37-43,52} of the western part of Japan, cattle have long been suffering from an enzootic nutritional disease, locally called "kuwazu (refuse eating)" or "kuiyami (cease eating)" disease. Affected cattle reveal almost the same symptoms as affected ruminants in Australia and other countries. Unfortunately, the etiology of this disease remained unknown for a long time. It was really regrettable that the disease had not come into question in the veterinary field until about the end of World War II. The occurrence of this disease was limited to certain areas, and affected animals gained recovery when moved from enzootic to healthful areas.

After World War II, active, systematic studies were initiated on the disease by FUKUSHIMA¹³ in 1951. At first it was presumed that the disease was due to the chronic poisoning of fluorine contained in drinking water¹³. FUKUSHIMA¹⁴ found that the clinical symptoms manifested in natural cases were almost the same as those shown by ruminants affected

Table 1. Animals Used, Onset of Symptoms, and Treatment

Animal number	Sex	Start of experiment			Weeks until onset of symptom	Days fed Cobalt deficient ration (until killed)	End of experiment (killed)			Treatment after onset of symptoms				
		Date	Age (months)	Body weight (kg)			Date	Age (months)	Body weight (kg)	Drug	Treatment		Days	
											Route	Dosage		From
N1	♀	•	•	•	•	•	Apr. 10 '51	72	•	•	•	•	•	•
E 4	♂	Jan. 8 '57	6	131	17	228	Aug. 23 '57	13	139	•	•	•	•	•
E 3	♂	//	6	130	23	514	June 5 '58	23	136	(Skim-milk powder)	PO	600g/day	Dec. 10 '57 ~ Feb. 11 '58	⁶⁴
										(Skim-milk powder Polyamin)	PO SC	500g/day 250cc/every other day	Mar. 4 '58 ~ June 3 '58	⁹²
E 2	♂	//	7	131	27	631	Mar. 2 '59	32	408	(Cobalt chloride)	PO	60mg/day	May 15 '58 ~ Mar. 1 '59	²⁹¹
E 1	♂	//	7	149	37	1,243	Nov. 14 '60	54	528	Vitamin B ₁₂	IM	600γ/day	Dec. 9 '58 ~ Jan. 20 '59	⁴³
										Vitamin B ₁₂	IM	500γ/twice a week	Jan. 24 '59 ~ Mar. 31 '59	⁶⁷
										(Copper sulfate)	PO	800mg/day	May 25 '59 ~ Aug. 18 '60	¹¹⁶
										(Cobalt chloride)	PO	40mg/day	Aug. 19 '60 ~ Nov. 14 '60	⁹⁸

Remarks: 1. N: natural case, E: experimental case, PO: per os, SC: subcutaneous, and IM: intramuscular.

2. Polyamin, a patent name, is an enzymic hydrolysate of casein.

3. Daily intakes of vitamins A and D were 40,000 and 6,000 IU for cattle E 1, E 2, and E 3 for 106, 382, and 112 days, respectively.

4. Anorexia was regarded as the first symptom.

Table 2. Cobalt-Deficient Basal Ration

Ration fed (kg/day)	Rice straw 0.4~3.0	Barley 0.3~5.0
Cobalt content (ppm)	0.029~0.072	0.004~0.15

Remarks: 1. The rice straw and barley used had been produced in enzootic areas in Hyogo Prefecture.
2. Rice straw was given at free choice.

Table 3. Composition of the Cobalt-Deficient Basal Ration Used

	TDN (%)	DCP (%)	Minerals				
			Ca mg%	P mg%	Fe mg%	Cu mg%	Co γ%
Barley	76.7	7.8	40	270	6	—	0.8*
Rice straw	37.1	1.0	405*	10.6*	12.9*	0.25*	3.5*

* Determined by chemical analysis carried out by FUKUSHIMA.

with cobalt deficiency, and demonstrated that affected cattle were cured with a small supplement of cobalt chloride. Confirming the distinct response of patients to the administration of cobalt salt *per os*, FUKUSHIMA^{14,18)} expressed his view that the cobalt contained in a ration had an important significance.

On the other hand, SAHASHI and IWAMOTO³⁹⁾, IWAMOTO²⁴⁾, and SAHASHI⁴⁰⁾ had been studying the disease biochemically since 1950 and concluded^{25,40)} that the disease was caused by vitamin B₁₂ deficiency in cattle in which the extermination of *Actinomyces* had been brought about by the toxic properties of fluorine contained in the running water.

Later FUKUSHIMA et al.^{13,16)}, and UESAKA et al.^{28,46,47)}, presented evidence against the fluorine hypothesis of SAHASHI and IWAMOTO^{34,35)}. Then UESAKA et al.^{45,46)}, stressed the importance of cobalt in the ration more than that of fluorine in the drinking water, and proved⁴⁹⁾ that the synthesis of vitamin B₁₂ by *Actinomyces* was not always parallel to its propagation. Moreover, FUKUSHIMA et al.^{17,22)} succeeded in inducing the disease to cattle by feeding them a cobalt deficient ration. Then they carried out therapeutic experiments, using cobalt drench, and obtained favorable results.

Literature on cobalt deficiency in ruminants is abundant, but there are relatively few papers^{7,32)} which deal in detail with histopathological studies under consideration. The studies reported herein were conducted to determine whether or not the histopathological findings of "kuwazu" disease in Japan were in accord with those of cobalt deficiency of ruminants. The results of investigation carried out on natural and experimental cases of cattle are described in this paper.

MATERIALS AND METHODS

The materials examined consisted of one cow naturally affected and four bull calves experimentally affected with "kuwazu" disease. The experimental animals were all fed a cobalt deficient ration (Table 2), produced in enzootic areas in Hyogo Prefecture, until they were slaughtered. The composition of the ration was shown in Table 3. After the onset of symptoms, all animals, except case E 4, were administered with skim-milk powder, cobalt chloride, and copper sulfate, or injected subcutaneously with polytamin (an injectable hydrolysate of casein) or intramuscularly with vitamin B₁₂ (Table 1).

All animals were slaughtered by total bleeding. Immediately after death, main organs and tissues were removed and fixed in 10 percent formalin solution, absolute alcohol, Carnoy's or Helly's fluid. Paraffin or frozen sections were prepared. Sections were stained with hematoxylin and eosin, azan stain, van Gieson stain for collagenous fibers, Gömöri's method of Bielschowsky-Maresch's silver impregnation for argyrophil fibers, and Sudan III for neutral fat. To some other paraffin sections, Prussian blue reaction was applied for the detection of iron-containing pigments. On the other hand, histochemical examination was performed by using Feulgen reaction and Bauer-Feulgen reaction for glycogen.

RESULTS

Since there was no fundamental difference between the findings of the natural case and those of the experimental ones, description was made summarizing the findings of both cases.

1. Clinical observation

The four bull calves fed the cobalt deficient ration manifested pica as the first symptom 17 to 37 weeks after the start of the experiment. At the same time, anorexia and constipation were observed in all calves, but one in which diarrhea was noted. The movements of the rumen and intestine were reduced; the waves of sound were less frequent and not so loud. The cow revealed listlessness, general emaciation, failure of growth, and rough hair coat. No animals shed their hair until late in the season. Scaliness of the skin, grinding of the teeth, lack of co-ordination in movement, marked anemia of the visible mucous membranes were observed. Hematological examination disclosed 40 to 50 per cent hemoglobin, 3 to 5 million red blood cells and 3 to 6 thousand white blood cells, with a relative decrease in granulocytes and an relative increase in lymphocytes. Anisocytosis and poikilocytosis were constant features and erythrocytes seemed to decrease somewhat in diameter.

An affected calf (E 2) was cured clinically by the use of a drench of cobalt chloride administered daily to supply 10 to 15mg of cobalt. Another affected calf (E 1), which had been injected intramuscularly with vitamin B₁₂ manifested a rapid response to this vitamin. After cobalt treatment, appetite and body weight increased, clinical symptoms disappeared, and hematological findings were improved in both calves. The red blood count increased to 7.5 and 7.7 millions, with increased hemoglobin and hematocrit values, in calves E1 and E2, respectively.

Administration of skim-milk powder and copper sulfate and injection of polytamin had no effect upon the animals.

2. Gross pathology

a. Animals (N 1, E 3 and E 4) administered with non-cobalt compound

The carcass revealed a picture of extreme malnutrition and emaciation. Thinness and paleness of musculature and lack of body fat were constantly observed. The subcutaneous tissue and musculature gave an impression of reduction of moisture.

The spleen was small and atrophic with a thickened capsule, which was slightly greenish-gray in color. Sections through the spleen was dark brownish-red in color; the follicles were atrophic and the trabeculae increased in number (Fig. 1).

The liver was small and yellowish-brown in color. Its parenchyma showed, in places, general (E 4) or partial (E 3) fatty degeneration and occasional congestion (E 4). The gall bladder (E 3 and E 4) was extremely extended, containing a mere viscous bile approximately 350 (E 3) and 450 cc (E 4) in quantity. The pancreas was light reddish-yellowish-gray in color. The kidneys were small; their capsule were peeled off with ease and a yellowish-grayish-brown surface was disclosed. Sections through the kidneys revealed the fairly well-demarcated cortex and medullary tissue. The parenchyma was slightly cloudy.

Table 4. Weight of Organs

Animal No.	Sex	Age (months)	Body weight (kg)	Heart (g)	Lung (g)	Spleen (g)	Liver (g)	Kidney (g)	Adrenal gland(g)	Pancreas (g)	Brain (g)	Thyroid gland(g)	Hypophysis (g)	Testis (g)
N 1	♀	72	281	•	•	•	•	•	•	•	•	•	•	•
E 4	♂	13	139	800	900	223	1,900	186 (R) 172 (L)	3.7 (R) 3.5 (L)	85	390	•	•	•
E 3	♂	23	136	700	950	210	1,724	166 (R) 139 (L)	4.6 (R) 3.8 (L)	93	345	8.9	1.4	55 (R) 44 (L)
E 2	♂	32	408	1,595	1,852	760	3,436	279 (R) 248 (L)	6.7 (R) 4.6 (L)	364	431	•	•	•
E 1	♂	54	528	2,240	2,920	1,100	4,140	320 (R) 372 (L)	8.2 (R) 9.2 (L)	443	424	15.9	3.9	366 (R) 349 (L)

Remarks: R: right, L: left.

The mucous membranes of the proventriculi were yellowish-white in color due to the disappearance of the pigment. Many pieces of rice-straw ingested were contained in the proventriculi, abomasum, and intestine. The wall of the whole digestive canal was thinner than normal.

Sections through the myocardium revealed an anemic appearance. The endocardium was smooth. The lungs were small and collapsed.

The lymph nodes were slightly edematous. The adrenal gland, testes, hypophysis, thymus, and thyroid gland were all atrophic.

b. Animals (E 1 and E 2) treated with cobalt chloride

The spleen (E 2) fixed in formalin solution was dark gray in color with moderately soft consistency. Its follicles were atrophic and indistinct. The liver showed subcapsular hemorrhages in places. Other postmortem findings were almost the same as those of healthy cattle, except congestion of the organs and tissues examined and relative diminution of the color of the mucous membranes of the proventriculi.

3. Histopathological findings

a. Animals (N 1, E 3, and E 4) administered with non-cobalt compound

Spleen: Thickening of the capsule and anemia was noted in the spleen. The pulp revealed a marked decrease in lymphocytes. The most conspicuous finding was hemosiderosis due to a marked increase in pigment granules which gave positive Prussian blue reaction (Fig. 2); so that the sections looked light blue by a naked eye. Pigment granules were phagocytized by many reticulum cells and macrophages, and found even in some Malpighian corpuscles. In many Malpighian corpuscles, the outer zone mostly disappeared (Fig. 2) and the inner zone was ill-defined. In general, active proliferation of argyrophile fibers was demonstrated in the spleen and often in the Malpighian corpuscles (Fig. 3).

Liver: The cytoplasm of many hepatic cells had fine granulated vacuoles; cell boundaries became more or completely indistinct. Especially in the centrilobular parts of the liver, the hepatic cells were swollen, stained dirty with hematoxylin and eosin (Fig. 4), and often lost their nuclei (N 1). In general, atrophy of the hepatic cells was so intense that most of these cells became small and round, and Disse's space was more (N 1 and E 3) or strikingly (E 4) dilated. Fatty degeneration of the hepatic cells was constantly present in varying degrees. It was pronounced in the peripheral or central part of some lobules (Fig. 5). A remarkable decrease in glycogen granules in the hepatic cells was noticed in every case. Neither hemosiderosis nor inflammatory cell infiltration was demonstrated in any case. Atrophy of argyrophile and collagenous fibers was conspicuous; argyrophile fibers disappeared from the midzonal areas of the lobules (Fig. 6). In general, degenerative changes were

more conspicuous in the natural case (N 1) than in the experimental ones (E 3 and E 4).

Kidneys: The kidneys were atrophic. The glomeruli were small, containing scanty erythrocytes (N 1 and E 4). A decrease in nuclei was noted. In the cavity of Bowman's capsule, reddish proteinous droplets or serous exudation was occasionally present (N 1). Urinary hyaline casts were often observed in the convoluted tubules in the cortex (N 1, E 3, and E 4).

Adrenal glands: Fatty degeneration was observed in the entire cortex in cases N 1 and E 4, in the zonae glomerulosa and media in case N 1 (Fig. 7), in the zona reticularis in case E 4. In the remaining case E 3, lipid droplets were very few in the cortex.

Pancreas: A decrease of zymogen granules and atrophy of the epithelial cells of the acini were predominant.

Proventriculi: Desquamation of the keratoid epithelium, apraxia or disappearance of melanin pigment, and active proliferation of germinal epithelial cells and fibrocytes in the lamina propria (Fig. 8) were observed. So that the thickness of the mucous membrane increased (E 3 and E 4). As a whole, the villi of the proventriculi were much more slender in these calves than in healthy animals. Focal necrosis in the upper part of the epithelial layer and atrophy of the muscular layer were observed. The border of the necrotic focus was sharply demarcated from better-preserved peripheral epithelial cells (Fig. 8).

Abomasum and intestine: Atrophy was a striking feature. The glandular elements of the mucous membrane were reduced in number (Fig. 9). The muscle fibers were slender, showing a wavy appearance in places.

Myocardium: Atrophy of the muscle fibers was generally observed. The muscle fibers were faded out, in places, into connective tissue.

Lungs: The pleura was thickened. Atrophy of the interstitial tissue, parenchyma, and diminution of elastic fibers were found.

Lymph nodes: The medullary and marginal sinuses were edematous. Atrophy of the germinal centers and decrease of lymphocytes were noted. Proliferation of reticulum cells and hemosiderosis could not be found. A marked decrease of red cells was noticed in the red lymphonodus.

Testes: The seminiferous tubules approximately 146 to 183 μ in diameter were small, far apart from each other, and strikingly atrophic. They had a lumen generally dilated due to a marked decrease, necrosis, and degeneration of germinal epithelial cells, which disappeared entirely in places (Fig. 10). Neither meiotic spermatogonia nor fat droplets in germinal epithelial cells were present (E 3 and E 4). Interstitial cells with fine-granular fat droplets were frequently observed.

Thymus (E 3 and E 4): Marked atrophy was noticed in the cortical substance, in which lymphocytes decreased strikingly in number or disappeared owing to the replacement by adipose tissue.

Thyroid gland (E 3 and E 4): Marked atrophy was noticed. The size of the follicle and the color of the colloid showed a considerable variation. The follicular epithelia with small nuclei were partly or wholly desquamated into the acinar cavity. No colloid-containing acini were observed in many of the follicles located mainly in the periphery of the gland (Fig. 11).

Tongue: Atrophy of the lingual tonsil and mucous glands was observed. The waxy degeneration in successive stages was often observed in the striated muscle showing inflation of muscle-fibers. Homogenization was seen in the sarcoplasm. In places, proliferation of fibrocytes was noted in the interstitial tissue. Desquamation and disappearance of epithelial cells of the mucous gland were predominant; each gland revealed a cobwebbed appearance.

b. Animals (E 1 and E 2) treated with cobalt chloride

Spleen: Congestion was a constant feature; the venous sinuses were distended and

filled with blood. A marked decrease in lymphocytes and striking atrophy of the follicles were the most conspicuous findings (E 2); many follicles were reduced markedly in size and often disappeared (E 2). Hemosiderosis was hardly observed. In places, many pigment granules stained by the Prussian blue reaction were found. Hemorrhage was often noticed in the trabeculae.

Liver: Marked swelling of the hepatic cells was generally observed. In places, marked subcapsular hemorrhages were observed (Fig. 12). The central veins were conspicuously distended with blood. On the contrary, the sinusoids were contracted due to pressure of the swollen hepatic cells. The cytoplasm of the hepatic cell was markedly granulated and stained faintly. Glycogen granules decreased strikingly in the midzonal areas of the lobules. No fatty degeneration of the hepatic cell was noted. No reaction of Kupffer's cells could be observed.

Kidneys: The glomeruli were enlarged and congested. There were distinct swelling and degeneration of the epithelial cells of the convoluted tubules, which often contained desquamated epithelial cells and reddish proteinous droplets. Bowman's capsule was often dilated, containing a serous exudate or proteinous droplets. Some of the nuclei of the tubules were pyknotic and occasionally disappeared. The free surface of the epithelial cell was destroyed and a bare nucleus was often observed (Fig. 13).

Myocardium: The myocardium generally showed congestion, but diapedetic hemorrhage (E 2) was rare in the intermuscular tissue. One case (E 2) revealed an early change of waxy degeneration diffusely, showing inflation of muscle-fibers and homogenization of the sarcoplasm due to the fusion of myofibrils (Fig. 14). No cellular proliferation in the interstitial tissue was observed.

Lymph nodes: Lymph stagnation, blood resorption, marked dilatation of the medullary and marginal sinuses, and the disappearance of the follicles were observed (Fig. 15).

Testes: The testes revealed an almost normal appearance (Fig. 16). The other organs and tissues examined showed no conspicuous changes, except congestion and slight atrophy of their cells.

DISCUSSION AND SUMMARY

The results obtained from these experiments indicate clearly the beneficial effect of a cobalt supplement to a cobalt-deficient basal ration and no beneficial effects of skim-milk powder, copper sulfate, and polytamin. Animals fed a cobalt-deficient ration manifested at first such symptoms as loss of appetite and body weight approximately 17 to 37 weeks after the start of the experiment. Affected animals revealed symptoms very similar to those observed in the case of cobalt deficiency^{1,2,9,10,33,36}. Such watery or serous discharge from the eyes as reported by REID³⁸, BEESON⁵, and STEWART⁴², however, was never encountered. It was difficult to find any specific clinico-anatomical findings of this disease.

The most specific effect of cobalt drench upon cattle (E 1 and E 2) was the recovery of appetite. These animals revealed a dramatic cobalt response. After cobalt administration, appetite increased in three to five days. Rumination and peristalsis became regular. The cattle recovered their normal appearance in less than two months, but it took about four months for pica and anemia to disappear. This dramatic response was demonstrated not only to administration of cobalt compounds but also intramuscular injection with vitamin B₁₂.

Postmortem and microscopical findings of both natural (N 1) and experimental (E 3 and E 4) cases administered with non-cobalt compounds revealed no fundamental differences. They included marked atrophy of all the organs and tissues examined and lack of body fat. The cut surface of the spleen of cattle E 3 and E 4 fixed in formalin solution was

brown in color, but that of the spleen of animal E 2 which had been administered with cobalt chloride *per os* daily for 291 days was dark gray. No inflammatory changes were noticed on the peritoneal surfaces of the abdominal organs. Histopathologically, marked hemosiderosis of the spleen and fatty degeneration of the liver were also reported in "nakurutitis" in Kenya and similar diseases in other countries^{10,36}. In all the materials examined by the authors, no hemosiderosis of the liver and kidneys could be demonstrated.

The red cell count was always low in all the animals examined. The anemia of this disease would appear to be of microcytic nature, and anisocytosis and poikilocytosis were constant features. The same results were reported by FILMER⁷ and MARSTON³⁰. No macrocytic anemia was observed. This finding was not in agreement with that of IWAMOTO²², who stressed that "kuwazu" disease might be a form of macrocytic anemia.

SAHASHI and IWAMOTO³⁹, IWAMOTO^{21,22}, and SAHASHI⁴⁰ stated that "kuwazu" disease seemed to occur from the deficiency of vitamin B₁₂ which Actinomycetes organisms produced. Their opinion was in accord with that of BECHER³.

Regarding hemosiderosis of the organs, there is a divergence of opinions among authors. MARSTON³⁰ reported that hemosiderosis of the spleen was found to be much less pronounced than that of the liver and pancreas, and that pigment deposits were rarely observed in the kidneys. On the contrary, FILMER⁷ stated that hemosiderosis of the spleen was more conspicuous than that of the liver and kidneys. KITANO²⁶ did not refer to hemosiderosis of the spleen in a goat affected with "kuwazu" disease. Such divergence of opinions concerning hemosiderosis may be due to the fact that different lesions developed in the different stages of the disease or to the difference in species of animals.

Further endocrinological and histological studies are necessary to clarify the mechanism of apraxia of pigment granules in the mucous membrane of the proventriculi.

The additional interesting findings observed are as follows.

- 1) Marked proliferation of germinal epithelial cells and fibrocytes in the lamina propria of the proventriculi.
- 2) Marked fatty degeneration of the adrenal cortex.
- 3) Marked degeneration, necrosis, or disappearance of germinal epithelial cells in the testes and the complete disappearance of meiosis in spermatogenesis.

The authors wish to think much of the findings of cattle E 1 and E 2. These cattle seemed to reveal the pathological changes of cobalt poisoning, but manifested no clinical symptoms, except considerably distinct polycythemia. The lesions located in the organs and tissues were more conspicuous in animal E 2 than in animal E 1.

CONCLUSIONS

Histopathological studies were performed on five cases, one natural and four experimental, of so-called "kuwazu" disease in cattle. The following conclusions were drawn.

1. The findings of the main organs and tissues examined were almost identical with those of cobalt deficiency in cattle.
2. Marked atrophy of all the organs and tissues examined was the principal feature of this disease.
3. The main lesions consisted of marked hemosiderosis of the spleen, striking fatty degeneration of the liver and the adrenal cortex, and reduction of the spermatogenic activity of the testes.
4. Apraxia of pigment granules in the epithelial cells and hyperplasia of germinal epithelial cells of the proventriculi were interesting findings of the disease.
5. An experimental animal was administered perorally with 60mg of cobalt chloride daily for 291 days. As a result, it seemed to reveal pathological changes of cobalt poisoning.

ACKNOWLEDGEMENTS

The authors are greatly indebted to Drs. S. UESAKA, professor at Kyoto University, K. HASHIZUME, professor, and the late Dr. R. NAKAYAMA, assistant professor, both at the University of Osaka Prefecture, for their advice and collaboration. Thanks are due to Mrs. L. K. WHITTEN, librarian of the Wallaceville Animal Research Station, New Zealand, who was kind enough to send many related papers to the authors.

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日本におけるウシのくわず病またはくいやみ病の組織病理的研究

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(昭和 38 年 3 月 10 日受付)

西日本の一部地方に、ウシのくわず病またはくいやみ病と俗称されている地域性栄養障害が発生していることは、古くから知られている。本病は、(1) 飼料および土壌中のコバルト含量が少ないこと、(2) 一定地区にのみ限定して発生すること、(3) 罹患したウシ（自然および実験例とも）を他の健康地帯に移動すると回復すること、さらに (4) 塩化コバルトの微量を経口投与すると、数日にして食欲が増進し、貧血その他の症状が急速に転快となることから、コバルトの欠乏症とみなされていた。

著者らは、このたび、本病が、諸外国において報告されているウシのコバルト欠乏症に一致するかどうかを究明する目的で、本病の病理学的研究を行なった。その結果、従来の諸家の知見にほぼ一致する所見を得、本病がウシのコバルト欠乏症にほかならないことを明らかにしたのみならず、さらに新知見を追加し得た。

本病は、全身の臓器および組織の高度の萎縮、脾

の著明な血鉄症、脾小体の萎縮、消失、肝細胞の荒廃、高度の脂肪変性およびグリコーゲン顆粒の減少または消失、副腎皮質における高度の脂肪変性、精巢の造精機能減退を特徴とする。血液検査では、小赤血球性貧血、赤血球の大小不同、または変形赤血球の出現が認められる。とくに興味ある知見は、各前胃粘膜の色素顆粒の減少または消失である。この事実の解明のためには、内分泌学的、組織化学的研究を要するものと考えられる。

さらに著者らは、くわず病の症状が出現してから、塩化コバルトを毎日 60 mg ずつ、291 日間経口投与したもの（牛 E2 号）において、病理学的に明らかに中毒の所見が認められたことを特筆したい。なおビタミン B₁₂ を毎日 500~600 γ ずつ 110 日間、筋肉内に注射し、引き続いて塩化コバルトを毎日 40 mg ずつ 98 日間、経口投与したもの（牛 E1 号）においても、病理学的に経度の中毒所見が認められた。

EXPLANATION OF PLATES

PLATE I

- Fig. 1. Spleen showing marked increase of trabeculae. Case E 4. $\times 2$.
Fig. 2. Spleen showing marked hemosiderosis and atrophy of follicle. Case N 1. Prussian blue reaction. $\times 100$.
Fig. 3. Active proliferation of argyrophile fibers in the spleen. Case E 4. Gömörie's silver impregnation method. $\times 100$.
Fig. 4. Marked degeneration of hepatic cells. Case E 4. Hematoxylin and eosin staining (H.-E.). $\times 200$.
Fig. 5. Midzonal fatty degeneration of lobules in the liver. Case E4. H.-E. $\times 30$.
Fig. 6. Atrophy and disappearance of argyrophile fibers in the liver. Case E4. Gömörie's silver impregnation method. $\times 100$.
Fig. 7. Marked fatty degeneration of zonae glomerulosa and media in the adrenal gland. Case N1. Sudan III stain. $\times 100$.
Fig. 8. Striking proliferation of germinal epithelial cells and fibroblasts in the lamina propria of villi of the proventriculi. H.-E. $\times 100$.

PLATE II

- Fig. 9. Reduction in glandular elements of the mucous membrane of the colon. Case E4. H.-E. $\times 100$.
Fig. 10. Marked atrophy of seminiferous tubules and disappearance of germinal epithelial cells. Case E4. H.-E. $\times 100$.
Fig. 11. Thyroid gland with follicles varying in size and containing scanty or no colloid. Case E3. H.-E. $\times 100$.
Fig. 12. Liver showing subcapsular hemorrhage and distended central veins with blood. Case E2. H.-E. $\times 100$.
Fig. 13. Kidney with swollen glomeruli and degenerated convoluted tubules. Case E2. H.-E. $\times 100$.
Fig. 14. Myocardium showing early change of diffuse waxy degeneration. Case E2. H.-E. $\times 100$.
Fig. 15. Mesenteric lymph node with dilated marginal sinus in which lymph stagnation is seen. Case E2. H.-E. $\times 100$.
Fig. 16. Seminiferous tubules exhibiting various stages of spermatogenesis. Case E2. H.-E. $\times 100$.