STUDIES OF VISCERAL PATHOLOGY IN FOWLS ON FISH OIL DIETS

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It has hitherto been considered in Japan that fish scraps or fish meal may produce hepatomegaly in laying hens, thus resulting in severe loss to the poultry raiser. For this reason many poultry feeding experiments with fish oil diets have been conducted to investigate the visceral pathology and to attempt to moderate or eliminate these pathological changes by supplementing the diets with so-called 'anti fatty-liver' substances.

SUGANO et al. conducted pathological investigations of chickens fed sardine oil. They found the deposition of acid-fast pigments in the livers and other organs. They reported that these changes corresponded to lesions observed in mink¹²⁾, ferrets, foxes, swine⁴⁾, and cats¹⁾ all of which had been fed diets deficient in vitamin E or containing highly unsaturated fatty acids. Although they noted the development of nodule-like lesions in the liver, they did not observe any true hepatomegaly. Later, KOYANAGI et al.^{8,9)} experimented with methods of preventing chickens and laying hens fed fish oil diets from so-called 'fatty hepatomegaly'. They⁸⁾ reported that ceroid-like pigment granules noted in the hepatic cells of birds disappeared entirely when the bird fed a diet supplemented with vitamin E, so long as the diet contained no iwashi acid. RIGDON et al. recognized the deposition of a ceroid-like pigment in the duodenal villi, liver, and spleen of chickens given gossypol.

In recent years, the deposition of acid-fast or ceroid-like pigment has been mentioned clearly in many reports as characteristic of the visceral pathology of fowls on fish oil diets or diets containing unsaturated fatty acid. Few of these reports, however, give details of any other morphological changes.

PAPPEHEIMER and GOETTSCH^{17),18)}, who performed experimental studies on E avitaminosis in chickens, pointed out a fairly high incidence of encephalomalacia among the affected chickens. Similar studies have been conducted by other authors. DAM^{2,3)} observed the occurrence of exudative diathesis in addition to encephalomalacia, and SCOTT et al. that of severe leg weakness and gizzard erosion, as well as exudative diathesis.

Since 1958, the present authors have been conducting pathological studies on the viscera of chickens and pullets on fish oil diets. The present report outlines the results of these pathological observations.

MATERIALS AND METHODS

Details of the materials used and experiments performed are given in Table 1. Ten-day-old white Leghorn chicks and 3- and 6-month-old pullets were used. The control groups were given either basal diets or basal diets plus varying amounts of sardine oil. The experimental groups were given fish oil diets supplemented with various

Table 1. Experimental Details

Exp. No.	Age in days	Sex	Duration of exp. in days	Date of autopsy	Number of cases	Organs examined	Percentage of fish oil in diet
1	10	♂	30	1/X '58	48	Liver	10%
2	10	♂	35	18/XI '58	35	All	8-15%
3	10	♂	35	25/XII '58	39	All	8-15%
4	10	♂	28	29/V '59	40	All	5-20%
5	10	♂	35	7/VI '60	49	All	5-15%
6	180	♀	210	10/IV '58	48	Liver	10%
7	84	♀	120	31/III '59	40	All	5%
Total					299	Liver alone:	96
						All organs:	203

kind of so-called 'anti-fatty-liver' substances, such as choline, methionine, greens, fresh or dried radish leaves, and vitamins E, B, and K. The duration of a feeding experiment was approximately 4 to 5 weeks for chickens and 4 to 7 months for pullets. At the end of each experiment, all the birds were autopsied. As is shown in Table 1, a total of 299 birds, including 211 chicks and 88 pullets, were divided into seven experimental groups.

All the experimental subjects were examined macroscopically and some specimens collected from them for microscopic examination. In two of the experimental groups, only the livers were collected. The majority of the specimens were fixed with 10 per cent formalin and a few with Carnoy's solution. Paraffin and frozen sections were prepared and the following stains and procedures were employed for morphological and histochemical examinations.

Paraffin sections: hematoxylin and eosin, Sudan III, Ziehl-Neelsen's carbol fuchsin, methylgreen, brilliant cresylblue, Mallory's hemofuscin method, Lillie's PFAS method, McManus' PAS method, Berlin-blue reaction, Bielshowsky's silver impregnation, and Schmorl's test for "Abnutzungspigment".

Frozen sections: Sudan III staining, Schultz's method for cholesterol, Fischler's method for fatty acid, and Kossa's method.

Fluorescence colors were observed in paraffin and frozen sections by ultraviolet irradiation.

RESULTS

There were distinct differences in visceral findings between the birds fed basal diets alone and those on fish oil diets. Most of the latter birds generally showed severe pathology despite the supplementation of their diets with so-called anti-fatty-liver substances, although they gave autopsy findings varying with the experimental methods used. The findings described in this report are, therefore, primarily confined to those of the birds on fish oil diets.

1. Macroscopic findings

At autopsy, the most notable changes were in the liver, duodenum, and adipose tissues throughout the body.

In the chicks, the liver invariably faded to a yellowish brown color and more or less increased in size. It was brittle and easily broken. In the duodenum, the mucosa was coarse and the serosa anemic in color. The duodenal lumen was expanded with a

quantity of dry, grayish-white material having the appearance of rice bran. The other parts of the intestines contained a yellowish gray material of the consistency of mud. In the majority of the chicks, the adipose tissues showed varying degrees of yellowish discoloration. Hemorrhages were noted in the subcutis, intermuscular areas, subperitoneum, gizzards, lungs, and livers in some of the chicks. No notable changes, however, were seen in the other portions of the body.

The changes in the viscera of the pullets were very similar to those of the chicks. These changes, however, were especially prominent in the 6th experimental group, presumably because this group had been fed a large quantity of fish oil supplemented to the diet for a long experimental period. These pullets exhibited distinct swelling of the liver and kidneys and frequent, fatal hepatic hemorrhages.

2. Histopathological observations

As observed before, there were apparent differences in histopathological changes between the groups on fish oil diets and the control groups given basal diets.

a. Histochemistry of pigments

Both in the chicks and pullets that had been fed fish oil diets, there were yellowish-brown or pale yellow pigments in sections stained with hematoxylin and eosin, especially of the liver, duodenum, and adipose tissues.

These pigments were previously reported by SUGANO et al., KOYANAGI et al., and RIGDON et al. as acid-fast pigments, ceroid, and ceroid-like pigments, respectively.

In the present study, various histochemical examinations were performed on such pigments, chiefly those contained in the liver and adipose tissue. The results obtained are presented in Table 2.

Table 2. Pigment Histochemistry

Method of staining	Section	Pigment	
		Large droplets	Fine droplets
Hematoxylin & eosin	Paraffin	Pale yellow, yellowish-brown or blue	Yellowish or indistinct
Sudan III	Paraffin	Yellowish-white or gray	Yellowish-white or gray
Sudan III	Frozen	Pale ocher	Reddish-brown
Mallory's hemofuscin	Paraffin	Bright red	Pale red
Methyl green	Paraffin	Bright green	Pale green
Brilliant cresyl blue	Paraffin	Blue	Pale blue
PFAS	Paraffin	+	+
PAS	Paraffin	+	+
PFAS following bromination	Paraffin	—	—
PAS following bromination	Paraffin	+	+
Ziehl-Neelsen	Paraffin	Dark red	Bright red
Berlin blue	Paraffin	—	—
Schmorl's test	Paraffin	—	—
Fluorescence	Paraffin	Pale yellow	Pale yellow
Fluorescence	Frozen	Greenish-yellow	Greenish-yellow

As may be seen in the table, the histochemistry of these pigments generally agrees with that of the "ceroid" pigment described by LILLIE et al. It was observed, however, that these pigments varied in characteristics even in the same organs. The results of these observations are given in detail in the description of each organ.

b. Findings in the organs

(1) Liver

In the birds fed basal diets, which contained no fish oil, the most noteworthy

finding was the deposition of fatty substances in the hepatic cells and the perivascular regions of the liver. This deposition was hardly detected in sections stained with hematoxylin and eosin, but was clearly observed in those subjected to Sudan III staining. The fat was deposited in the cytoplasm of the hepatic cells, forming fine, yellowish-brown granules. In the perivascular regions the fat was almost always observed in a form of large droplet and stained reddish yellow. Fat droplets varied in amount even in birds showing obscure deposition of fat in the hepatic cells. In addition, formation of lymph follicles, occasionally developed into a prominent perivascular infiltration of round cells, was often noted both in the chicks and the pullets.

The primary pathology in the birds fed fish oil diets consisted of the deposition of yellowish pigment in the periportal regions, hepatic cells, and sinusoids and the occurrence of regressive lesions in the parenchyma. These regressive lesions appeared as necrobiotic changes in the hepatic cells, syncytium formation, and eosinophilic degeneration.

Distinct changes were usually observed in the liver, although they varied with the experimental procedures. They were most obvious in the chicks of the 5-week experimental groups (exp. 2 and 3) and the pullets of the 6th experimental group. They were not so obvious in the chicks of the 28- and 30-day groups (exp. 1 and 4) and the pullets of the 120-day group (exp. 7).

(a) Findings in chicks

In sections stained with hematoxylin and eosin, the sinusoidal endothelia showed marked swelling and the lumen contained scattered cells, some of which had bronze-colored pigment in them. A few or more hepatic cells formed a syncytium. Such syncytia were occasionally scattered in the parenchyma. The cytoplasm of these cells contained yellowish-brown or bronze-colored pigment with regressive changes in the nuclei. In these lesions, atrophy occurred also to some extent, and interstices were formed between these lesions and the surrounding parenchyma. Such lesions were especially prominent in the periportal areas, where large cells with yellowish-brown pigment were arranged around the portal vein, showing an appearance of layers of pavement stones (Figs. 2 and 5).

In addition, necrobiotic foci with karyopyknosis, hyperchromatosis of the nuclear wall, karyorrhexis and karyolysis were observed, as well as occasional eosinophilic degeneration of the parenchyma. The parenchyma was entirely destroyed in these areas as a result of these changes (Fig. 6).

With the carbol-fuchsin method the deposition of the pigment was much more distinct. In such cases as presenting only a slight deposition, the pigment was usually observed in coarse droplets in the Kupffer cells. In cases with more marked deposition, it was also present in coarse granules like raspberries in the neighboring hepatic cells and in the nodule-like lesions. Diffuse deposition of fine granules of the pigment was also noted in the hepatic cells in some cases. The most outstanding change was the accumulation of large pigment-laden cells in the perivascular regions.

(b) Findings in pullets

Marked changes were noted in one of the pullet groups (exp. 6). In the majority of the birds of this group, circumscribed lesions were observed throughout the hepatic parenchyma. The hepatic cells were combined into large or small syncytia, which were separated from the surrounding parenchyma by empty spaces. Dark blue or bronze-colored pigment granules were deposited in these lesions, which were scattered throughout the parenchyma (Fig. 1). In some cases, widespread lesions occupied the majority of the parenchymal area, giving a map-like appearance (Fig. 3). However, neither proliferation of lattice fibers nor fibrosis was present in these lesions (Fig. 4).

In sections stained with Sudan III, the hepatic cells were filled with sudan-positive granules, frequently consisting of coarse red droplets, in addition to fine, yellowish-brown granules and dark yellow or ocher pigment. Eventually, distinct sudan-positive substances appeared in the parenchyma, except in circumscribed lesions. On the other hand, the substances deposited in the lesions usually stained pale to dark brown. In sections treated with the carbol fuchsin method, there were dense depositions of dark, reddish or pinkish pigment granules in these lesions, while the other areas were only faintly stained.

Some of the pullets manifested fatty changes. In these changes, there were diffuse neutral fat droplets in the hepatic cells, but no circumscribed lesions or deposition of acid-fast pigment. These changes were most frequent in the 7th experimental group, in which other morphological changes were generally slight.

(2) Intestines

Necrobiosis was seen in the mucosal epithelium of the duodenum in the majority of the cases with hyperchromatosis of the nuclear wall in the villous areas (Fig. 12). Desquamation was also fairly common in the epithelia. Round shed cells and debris of these cells filled the lumina of the canals. Edematous changes were noted in the villi of some cases. In most cases, large cells with droplets of yellowish pigment were found in the perivascular regions or in the top portions of the villi. Pigment substances were also present in lymph follicles of the submucosa in some cases. Though histiocytic and lymphocytic infiltrations were also noted in the villi and subserosa of some cases, the majority of these cases were free from any noticeable cell infiltration in the tunica propria or submucosa.

The pigments appeared more distinct in sections treated with Ziehl-Neelsen's acid-fast staining. They were seen as dark-red droplets in large cells in the villi. In some cases, they were scattered in masses in the mucosal follicles (Figs. 10 and 11). Occasionally, a diffuse deposition of fine granules was noted in the mucosal epithelia.

In some cases there was desquamation of the mucosal epithelia and a slight deposition of acid-fast pigment in the tunica propria of the jejunum, ileum, and cecum. There were, however, no other significant changes in these areas of the intestines.

The most noteworthy of these changes was necrobiosis in the mucosal epithelium of the duodenum. It was present in almost all the experimental chicks, except those of the four-week group (exp. 4). It was also observed in those pullets fed unsupplemented fish oil diets. There were no other notable changes in the pullet intestines.

(3) Adipose tissues

In most cases, a deposition of yellowish pigment was noted in the adipose tissues of the entire body; viz., in those of the subcutis, subserosa of the mesentery, bursa fabricii, proventriculus, gizzard, heart, spleen, liver, and kidney. The changes in these tissues may be summarized as follows.

(a) Yellowish or reddish-yellow, intra- or extracellular pigment granules were scattered among mature fat cells, either singly or in clumps. The single cells had the appearance of young fat cells, with a flat nucleus in the periphery of the round yellowish droplet. The pigmented groups were scattered with round, oval, and irregular-shaped nuclei or nuclear fragments. It was felt that the irregular-shaped nuclei might indicate a regressive change. In addition, there were syncytia formed by pigment-containing cells. In all the birds, the majority of the pigmented cells were located in the vicinity of the adventitia of the capillaries. The single cells particularly appeared to have developed from the adventitial cells. The existence of capillaries was obscured when these cells increased in number and accumulated in clumps.

(b) In some cases, a diffuse yellowish-gray pigment replaced the normal fat in mature fat cells. These cells were scattered among normal mature fat cells.

(c) Diffuse deposition of the pigment was noted in some areas of the adipose tissues. In these areas, the pigment cells were grouped together among mature fat cells, giving the appearance of a giant cell. In addition, there was a marked proliferation of histiocytic cells in the interstices between these pigment cell masses and scattered small, round vacuoles in these lesions. As the small vacuoles were proved to be neutral fat, it was presumed that these areas might have resulted from abnormal development of adipose tissue (Fig. 14).

(d) Hyperemia and dilatation of the capillaries, as well as hemorrhages, were noted in some cases, accompanied by a slight proliferation of heterophils in or near the lumens of the capillaries. No marked steatitis, however, was noted at the sites of deposition of the yellowish pigment, except for the areas with some scattered round cells and plasma cells.

(4) Spleen

No significant changes were noted in the general morphological examination. In sections treated with Ziehl's method, some scattered pigmented cells were detected in the red pulp. Such cells, however, were not observed in sections stained with hematoxylin and eosin.

(5) Kidneys

In some cases, there were necrobiotic changes, especially karyopyknosis, in the tubular cells, in addition to a proliferation of round cells and stagnation in the interstices. No significant changes were noted, however, in most cases. The following results were obtained from 139 cases.

Degenerative changes	13 (9%)
Cell infiltration	32 (23%)
Stagnation	4 (3%)
No significant changes	90 (65%)

In most cases there was yellowish pigment in the perivascular areas of the renal interstices.

(6) Heart

In many cases, there was a slight infiltration of histiocytes and lymphocytes in the intermuscular tissues and the subepicardium. Rarely, hemorrhages and hyalinosis were noted in the cardiac muscle. There was only an occasional deposition of the pigment. The pigment was present in coarse droplets in macrophage-like cells in the perivascular areas of the subendocardium in some cases.

(7) Proventriculus

Some cases exhibited necrosis and desquamation of the mucosal epithelia, as well as mucous secretion or infiltration of heterophils in the tunica propria. There was no deposition of pigment.

(8) Gizzard

There was a high incidence of hemorrhage, heterophilic infiltration, and hyperkeratosis in the mucosa. Edematous and hyalinizing changes were also noted in the muscular layers in some cases. There was no deposition of pigment in this organ either.

(9) Skeletal muscle

No significant changes were observed in the skeletal muscle, except hyalinosis and fragmentation which occurred in some cases.

(10) Bone marrow (femoral)

The bone marrow was examined only in 49 birds of the 5th experimental group. It had been taken from the femoral bones. There was a deposition of the pigment in all the birds, except those on diets supplemented with vitamin E. The pigment was accumulated in large or small quantity in the interstices of the adipose tissues.

(11) Brain

In most cases there was a slight accumulation of cells in the perivascular spaces of the cerebrum (Fig. 8) and cerebellum. In some cases, there was fibrinous thickening of the vascular walls. Some of the cells in the perivascular interstices contained yellowish pigment. There was occasional evidence of nerve cell degeneration. A few perivascular hemorrhages were present in the cerebrum. The evidence of new encephalomalacia, primarily in the medulla of the cerebellum of chicks, was especially noteworthy. Although no fat granular cells appeared in this region, edematous changes in the neighboring tissues, especially in the granular layer, and scattered myelin corpuscles were noticed (Fig. 7).

In 50 chicks, the incidence of the lesions described above was as follows.

Encephalomalacia	3 (6%)
Perivascular cell accumulation	7 (14%)
Degenerative changes in nerve cells	4 (8%)

The yellowish pigments mentioned above were stained reddish-purple by Ziehl-Neelsen's method. They were distinctly obvious in coarse droplets in macrophage-like cells among the cells accumulated in the perivascular regions (Fig. 9). Fine pigmented granules were also present in the lesions of encephalomalacia and in the capillary walls in the cerebellum.

3. Relationship between supplementation and visceral pathology

In the present experiment, various drugs and other substances were examined for their effect on the prevention of visceral pathology when added to fish oil diets. The supplements used were choline, methionine, vitamin E, rape scraps, extracts, extract residua, and fresh or dried radish leaves. They were given singly or in combination of two or three at various proportions.

Effectiveness was compared among these supplements in terms of the incidence of such visceral pathology as the deposition of acid-fast pigments and morphological changes in the liver, duodenum, and adipose tissue of fowls fed fish oil diets to which these supplements had been added. Although statistical comparison of the effects is complicated by a number of variables, such as the quantities of fish oil and supplements used, length of experimental period, age and number of subjects, it should still be possible to infer some general tendency from the results which are given below.

(1) Findings in the liver

In the liver, the deposition of acid-fast pigments in the hepatic cells, sinusoids and perivascular portions, and the presence of nodule-like lesions or necrotic foci were taken into consideration in the comparison of the effectiveness of the preventive agents. The results are shown in Table 3.

Pigment deposition was detected in nearly all the cases and the formation of nodule-like lesions in about one half of the cases given unsupplemented fish oil diets. As is clear in the table, there was little difference in the percentage of these lesions between the cases given fish oil diets supplemented with choline, methionine, and various greens and those given unsupplemented fish oil diets. There was a trend toward mitigation or disappearance of the lesions in the cases given fish oil diets supplemented with vitamin E, or vitamin E plus the substances listed above. The effect of vitamin E seemed to have been influenced by the quantities of these substances given. It was felt that any

Table 3. Results of Supplementation in Terms of Liver Pathology

Supplement*	Total number of cases	Acid-fast pigment		Nodule formation	
		Number of cases	Percent to total	Number of cases	Percent to total
None	54	53	98	28	52
Choline	35	35	100	26	74
Methionine	7	7	100	4	57
C, M	42	40	95	21	50
C, radish leaves	11	11	100	8	73
Dried radish leaves	7	7	100	2	29
Rape scraps	8	8	100	5	63
Rape extracts	11	11	100	9	82
10 mg%E, C, M	5	4	80	1	20
5 mg%E, greens, C, M	5	5	100	2	40
5 mg%E, greens	5	4	80	2	40
5 mg%E	9	5	56	4	44
10 mg%E	5	0	0	1	20
>10 mg%E	6	0	0	0	0
>10 mg%E, C	6	0	0	0	0
>10 mg%E, C, M	6	0	0	0	0
>10 mg%E, C, M, K, B	6	0	0	0	0

* C: choline; M: methionine; B, E, and K: vitamins B, E, and K.

Table 4. Results of Supplementation in Terms of Duodenal Pathology

Supplement*	Total number of cases	Acid-fast pigment		Necrosis in epithelia	
		Number of cases	Percent to total	Number of cases	Percent to total
None	42	37	88	26	62
Choline	16	11	69	14	88
Methionine	7	7	100	4	57
C, M	43	30	70	29	67
Rape scraps	8	2	25	6	75
Rape extracts	11	1	9	6	55
Dried radish leaves	7	6	86	7	100
5 mg%E, greens	10	7	70	5	50
10 mg%E	5	4	80	4	80
10 mg%E, C	5	0	0	0	0
10 mg%E, C, M	5	5	100	2	40
>10 mg%E, C, M	6	3	50	2	33
>10 mg%E, C, M, K, B	6	1	17	0	0

* See Table 3.

effect could hardly be expected when only small quantities of vitamin E were given.

(2) Findings in the duodenum

The deposition of acid-fast pigment and necrobiotic changes in the epithelia were the primary criteria of pathology in this organ.

As is shown in Table 4, these lesions were observed in a large number of cases given unsupplemented fish oil diets. Deposition of pigment occurred in 88% of these cases and necrobiotic changes in 62%.

The relationship between the development of lesions in the duodenum and the amount of supplements used appeared to be similar to the relationship observed in the liver. Reduction was noted in the number and severity of these lesions in the cases fed a diet supplemented with vitamin E. It was considered noteworthy, however, that these lesions were not entirely eliminated after the addition of vitamin E, although they became generally less severe after it.

(3) Findings in the adipose tissues

Deposition of acid-fast pigment was the primary criterion for pathology in these tissues.

Although the number of cases observed was small, deposition of pigment was noted in all the cases given unsupplemented fish oil diets. The effect of supplement on these tissues was similar in kind and degree to that on the liver and duodenum previously noted.

(4) Comparison of the severity of the visceral lesions

The results obtained from the 4th experimental group were considered the most

Table 5. Results of Supplementation in Terms of Deposition of Acid-fast Pigment in Adipose Tissue

Supplement*	Total number of cases	Deposition of acid-fast pigment	
		Number of cases	Percent to total
None	24	24	100
Choline	6	6	100
Methionine	6	4	67
C, M	15	11	73
Rape scraps	8	5	63
Rape extracts	11	10	91
5 mg%E, C, M, greens	5	5	100
5 mg%E, greens	5	2	40
5 mg%E	9	2	22
10 mg%E	5	0	0
10 mg%E, C, M	5	5	100
> 10 mg%E	4	1	25
> 10 mg%E, C	6	0	0
> 10 mg%E, C, M	6	0	0
> 10 mg%E, C, M, K, B	6	0	0

* See Table 3.

Table 6. Degree of Severity of Visceral Lesions in the 4th Experimental Group

Type of oil	Supplement*	Total number of cases	Degree of severity		
			Severe	Moderate	Mild or none
Fresh sardine oil	None	5	—	5	—
	C, M	5	—	5	—
	5 mg%E, greens	5	—	2	3
	10 mg%E	5	—	—	5
Oxidized sardine oil	None	5	—	5	—
	C, M	5	2	3	—
	5 mg%E, C, M, greens	5	—	2	3
	10 mg%E, C, M	5	—	—	5

* See Table 3.

suitable for this comparison. As is shown in Table 6, the lesions produced in birds fed the fish oil diet with addition of choline and methionine were as severe as, and sometimes even severer than, those observed in the control group given only fish oil. On the contrary, those birds to which a diet supplemented with vitamin E had been given produced slight or sometimes no lesions at all.

From the foregoing results, it is assumed that the addition of vitamin E to fish oil diets may be most beneficial for the prevention of the visceral pathology.

DISCUSSION

As was mentioned at the beginning of this paper, there have been many reports on visceral changes in fowls given fish oil or vitamin E deficient diets. The primary visceral changes described were encephalomalacia (PAPPENHEIMER and GOETTSCH^{17,18}), and encephalomalacia, exudative diathesis, and gizzard erosion (DAM^{2,3}) and SCOTT et al.²²). No description, however, was made on pigment deposition in any of those reports. Deposition of ceroid pigment and other histological changes were reported in the liver of chickens given fish oil diets by KOYANAGI et al.^{8,9}) It is of interest to note that RIGDON et al.²⁰) observed deposition of a ceroid-like pigment in viscera, especially in duodenal villi, of chickens given gossypol. They²⁰) also reported desquamation and degenerative changes in the mucosal epithelium of the duodenum, but detected no significant changes in the liver or spleen.

It is perhaps natural that the changes noted by the above authors should differ in appearance among them inasmuch as they were produced by different diets. It is felt, however, that these changes probably were of similar etiology. The changes observed were produced by diets containing excessive amounts of highly unsaturated fatty acids or by diets deficient in vitamin E. Therefore, it may be said that these changes varied only quantitatively. The deposition of acid-fast pigments and the occurrence of degenerative changes in the organs and tissues of the entire body may be presumed to have resulted from such diets.

The histochemical nature of the pigment in man and many animals has already been clarified thoroughly by many investigators^{5,7,10,11,15,16,19,21,27,28}). In the present experiments, deposition of such pigments as this occurred in almost all the organs and tissues, though in varying degree. The histochemistry of these pigments was reported previously by the present authors¹³), who found these pigments to be similar to the acid-fast pigment, or "ceroid", first observed and reported in rats by LILLIE et al.^{10,11}) The authors now assume, however, that this may not be entirely true since the pigments vary both qualitatively and quantitatively in each case. This variation has already been reported by the present authors¹⁴) in the adipose tissues of swine affected by yellow fat disease. In short, these substances are produced by ingestion of diets containing excessive amounts of unsaturated fatty acids. It is to be expected that they should be derived from fatty substances and that the observed qualitative differences should have resulted from varying degrees of oxidation.

In reference to the site of such pigment deposition, it was noteworthy that in addition to the adipose tissue, the liver and duodenal mucosa almost always contained these pigments. The deposition was especially prominent in the liver. This seemed strange in comparison with the deposition produced in swine under similar conditions. In explanation, it is considered probable that fat is normally deposited in the liver of fowls since deposition of fatty substances has almost always been observed in the perivascular areas and in the hepatic cells of fowls given diets entirely devoid of fish oils. From

these observations, it appears quite reasonable that marked deposition of the pigment should appear in the liver, particularly in the perivascular area, of fowls fed fish oil diets. This opinion is also supported by the findings in the adipose tissues.

In view of the results mentioned above, the pattern of pigment deposition may be interpreted as the result of abnormal development of adipose tissue. Moreover, it is assumed that even unsaturated fatty acid may form adipose tissue, and that disturbance in the normal process of fat metabolism may induce intra- or extracellular pigmentation. Eventually, some amount of this pigment may be ingested as a foreign body by histiocytes.

It is inferred from the foregoing statement that the unsaturated fatty acid may not merely be ingested by macrophages, but also be transformed to fat cells.

Some of the fish oils may of course contain certain elements which produce irritation or toxicity. In consequence, such regressive changes as degeneration, necrosis, and inflammation may appear in the parenchymal organs and the adipose tissues. SCOTT et al.²²⁾ reported gizzard erosion in vitamin E deficient chickens. In the present experiment, however, the primary pathology observed in the gizzard was mucosal hemorrhage and heterophil infiltration or hyperkeratosis in the horny layers. These pathological changes may be closely related to the ingestion of fish oil.

Necrobiotic changes were observed in the tubular epithelia of the kidneys. The incidence of such changes, however, was lower than was anticipated, and the majority of cases showed no significant pathology.

Detailed reports on the occurrence of nutritional encephalomalacia in chickens have already been presented by PAPPENHEIMER and GOETTSCH^{6,17,18)} and WOLF and PAPPENHEIMER²⁶⁾. In Japan, the occurrence of similar lesions has been reported by TAJIMA²⁴⁾ in natural cases and by USHIJIMA et al.²⁵⁾ in experimental birds given fish oil diets. As similar changes were also observed in the present experiments, it seems clear that the occurrence of these lesions was closely related to the ingestion of fish oil or to vitamin E deficiency. It is to be regretted, however, that there was no definite indication of the pathogenesis of these lesions, although fine granules of acid-fast pigment were observed on the walls of capillaries in and around the lesions.

It has been mentioned previously that supplementation of fish oil diets with choline, methionine, and various greens is ineffective in the prevention of lesions, so far as the results of histopathological examination are concerned. Some tendency toward inhibition or mitigation of such lesions was observed in birds which had been fed a diet supplemented with vitamin E, although it varied in the degree of such effect with the quantity of supplementation. It is considered that the supplementation of vitamin E may be beneficial to prevent fowls from such lesions.

SUMMARY

Histopathological studies were carried out on 299 chicks and pullets which had been used in feeding experiments with fish oil. In them, the pathogenesis of the pigment deposition and the pathomorphological changes were investigated, as well as the effects of various drugs and other supplements upon the prevention of the development of visceral lesions. The primary results are as follows.

1. Deposition of various acid-fast pigments was noted in various organs and tissues of the entire body, especially in the liver, adipose tissue, duodenal mucosa, and bone marrow.

The histochemistry of most of these pigments was largely similar to that of "ceroid"

reported by LILLIE et al.^{5,11}), although the pigments varied considerably in appearance and characteristic.

2. The pigment deposition in adipose tissues, livers, and other organs should not be regarded as a merely regressive change. It should be considered positively as a result of the disturbance of fat metabolism. Consequently, it was considered that the lipoblasts might be assumed as the source of the pigment cells other than macrophages.

3. In the experiments, regressive changes were also noted in the viscera. They consisted of necrobiosis and dissociation in the hepatic cells, syncytial formation and eosinophilic degeneration in the liver, necrobiosis and desquamation in the epithelium of the duodenal mucosa, and occasional necrobiosis in the kidneys.

4. In the brain, new lesions of encephalomalacia were observed in the medulla of the cerebellum in some cases. In addition to degeneration of nerve cells in the cerebrum, perivascular cell accumulation and fine hemorrhagic lesions were noted in a small number of cases.

5. From the results of the histopathological examinations, it was concluded that, of the various supplementary substances added to the fish oil diets, only vitamin E could be said to have produced many effective preventive results.

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REFERENCES

- 1) COFFIN, D. L. and HOLIZWORTH, J. (1954): *Cornell Vet.*, **44**, 63~71.
- 2) DAM, H. (1944): *J. Nutr.*, **27**, 193~211.
- 3) DAM, H. (1944): *J. Nutr.*, **28**, 297~302.
- 4) DAVIS, C. L. and GORHAM, J. R. (1954): *Amer. J. vet. Res.*, **15**, 55~59.
- 5) ENDICOTT, K. M. and LILLIE, R. D. (1944): *Amer. J. Path.*, **20**, 149~153.
- 6) GOETTSCH, M. and PAPPENHEIMER, A. M. (1936): *J. biol. Chem.*, **114**, 673~687.
- 7) GYÖRGY, P. and GOLDBLATT, H. (1942): *J. exp. Med.*, **75**, 355~368.
- 8) KOYANAGI, T., MIKAMI, S., ONO, K. and YOSHIDA, M. (1959): *Jap. J. zootech. Sci.*, **29**, 372~376 (in Japanese with English summary).
- 9) KOYANAGI, T., MIZUTANI, K. and KURODA, R. (1960): *Jap. J. zootech. Sci.*, **31**, 68~71 (in Japanese with English summary).
- 10) LILLIE, R. D., DAFT, F. S. and SEBRELL, W. H. (1941): *Publ. Hlth. Rep. (Wash.)*, **56**, 1255~1258.
- 11) LILLIE, R. D., ASHBURN, L. L., SEBRELL, W. H., DAFT, F. S. and LOWRY, J. V. (1942): *Publ. Hlth. Rep. (Wash.)*, **57**, 502~508.
- 12) MASON, K. E. and HARTSOUGH, G. R. (1951): *J. Amer. vet. med. Ass.*, **119**, 72~75.
- 13) MIURA, S. and OHSHIMA, K. (1960): *Jap. J. vet. Sci.*, **22**, Suppl., 442 (in Japanese).
- 14) MIURA, S., OHSHIMA, K., NUMAKUNAI, S., KIKUTA, T. and NUMAJIRI, Y. (1963): *J. Fac. Agr. Iwate Univ.*, **6**, 269~284 (in Japanese with English summary).
- 15) MURAI, H. (1958): *Chikusanno Kenkyū*, **12**, 247~254 (in Japanese).

- 16) NISHIO, S. (1962): *Bull. nat. Inst. agr. Sci.*, **G21**, 89~104 (in Japanese).
- 17) PAPPENHEIMER, A. M. and GOETTSCH, M. (1931): *J. exp. Med.*, **53**, 11~26.
- 18) PAPPENHEIMER, A. M. and GOETTSCH, M. (1933): *J. exp. Med.*, **57**, 365~371.
- 19) PAPPENHEIMER, A. M. and VICTOR, J. (1946): *Amer. J. Path.*, **22**, 395~413.
- 20) RIGDON, R. H., CRASS, G., FERGUSON, T. M. and COUCH, J. R. (1958): *Arch. Path.*, **65**, 228~235.
- 21) SCHMIDT, R. (1953): *Virchows Arch. path. Anat.*, **323**, 123~132.
- 22) SCOTT, M. L., HILL, F. W., NORRIS, L. C., DOBSON, D. C. and NELSON, T. S. (1955): *J. Nutr.*, **56**, 387~402.
- 23) SUGANO, S. and INOUE, M. (1957): *Jap. J. vet. Sci.*, **19**, Suppl., 47 (in Japanese).
- 24) TAJIMA, M. (1954): *J. Japan vet. med. Ass.*, **7**, 6~10 (in Japanese).
- 25) USHIJIMA, J., MATSUKAWA, K. and ICHIKAWA, T. (1962): *Jap. J. vet. Sci.*, **24**, Suppl., 483 (in Japanese).
- 26) WOLF, A. and PAPPENHEIMER, A. M. (1931): *J. exp. Med.*, **54**, 399~405.
- 27) WOLF, A. and PAPPENHEIMER, A. M. (1945): *J. Neuropath. exp. Neurol.*, **4**, 402~406.
- 28) YOKOYAMA, K. and ISHIDA, K. (1957): *Tohoku J. agr. Res.*, **8**, 113~119.

魚油給与鶏の内臓病変に関する研究

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魚油給与実験ヒナおよび成鶏 299 例の死体材料について、病理学的研究を行なった。従来、多くの人により観察されている色素沈着について再吟味する一方、形態学的変化に関する病理発生、各種防除剤の効果などについて検討した。

1. 全身の諸組織、特に肝、脂肪織、十二指腸粘膜および骨髓に、耐酸性色素物質の沈着が見られる。本色素物質の大多数のものは、各種の組織化学的所見を総合すると、LILLIE らの言う“ceroid”に一致するものである。しかし、その性状は必ずしも一定不変のものではなく、種々の段階があることを認めなければならない。

2. 脂肪組織あるいは肝における色素沈着については、単にこれを退行的な変化とみなす以外に、一部脂肪織生成上の異常過程と解したい。従って、この場合、ある積極的な意味を加味し、色素保有細胞

の起源については、一般的に考えられている食細胞のほかに、脂肪芽球の発生で重要視されている血管外膜細胞をも、考慮する必要がある。

3. 実験例においては、以上の色素沈着とともに、体腔臓器における退行的病変も見られた。肝実質の不全壊死および解離、合胞体形成、好酸変性、十二指腸粘膜上皮の不全壊死、剝離、一部の尿細管上皮の不全壊死などがそれである。

4. 魚油給与ヒナの一部において、小脳髓体に新鮮軟化巣の存在が注目された。その他、少数例において、大脳神経節細胞の退行像、囲管性細胞増数、小出血などが認められた。

5. 魚油飼料に添加して各種防除剤の効果を試験した結果、内臓病変の発現防止上、病理組織学的に最も有効と認められたものは、ビタミン E のみであるといえる。

EXPLANATION OF PLATES

PLATE I

- Fig. 1. Pullet. Nodule-like lesions in the hepatic parenchyma. Hematoxylin-eosin staining (H-E). $\times 300$.
- Fig. 2. Chick. Perivascular deposition of acid-fast pigment in the liver. Ziehl-Neelsen's acid-fast staining (Z-N). $\times 300$.
- Fig. 3. Pullet. Widespread pigment deposition in the hepatic parenchyma, showing map-like appearance. Z-N. $\times 300$.
- Fig. 4. Pullet. Same section as Fig. 3, showing lattice fibers in the pigment deposition. No productive figures are seen. Silver impregnation method. $\times 300$.
- Fig. 5. Chick. Yellowish pigment deposition in the liver (upper half of figure). H-E. $\times 1,200$.
- Fig. 6. Chick. Same section as Fig. 5. Degenerative changes in the hepatic cells, especially eosinophilic degeneration. H-E. $\times 1,200$.

PLATE II

All figures show lesions in chicks.

- Fig. 7. New lesion of encephalomalacia in the cerebellum. H-E. $\times 300$.
- Fig. 8. Perivascular cell accumulation in the cerebrum. H-E. $\times 300$.
- Fig. 9. Acid-fast pigment cell (arrow) among accumulated cells in the perivascular portion of the cerebrum. Z-N. $\times 1,200$.
- Fig. 10. Acid-fast pigment deposition in the duodenal villi. Z-N. $\times 300$.
- Fig. 11. Acid-fast pigment deposition, particularly in the upper portions of the duodenal villi. Z-N. $\times 300$.
- Fig. 12. Hyperchromatosis of the nuclear wall in the mucosal epithelium of the duodenum. H-E. $\times 1,200$.
- Fig. 13. Adipose tissue in the intestinal serosa. Acid-fast pigment cells accumulated in the interstices between mature fat cells. Z-N. $\times 300$.
- Fig. 14. Adipose tissue in the bursa fabricii. Diffuse deposition of yellowish pigments and proliferation of histiocytic cells. H-E. $\times 300$.