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Anatomy

Histological Assessment of Clarias Gariepinus' Liver and Kidney: An Ecotoxicological Evaluation of Commercial Fish Farm in Ogbogoro, Rivers State Nigeria

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Abstract: This study examined the ecotoxicological evaluation of commercial fish pond in Ogbogoro, Port Harcourt, Rivers State Nigeria using the histological assessment of Clarias gariepenus, using African Aquaculture Centre (ARAC) as a reference site. The sampling involved harvesting of twenty table sized fish from Ogbogoro commercial fish pond and ten table-sized of the same species from ARAC. The histological assessment involved the determination of the qualitative and semi-quantitative analysis of the Liver and kidney of the harvested fishes. Histological alterations observed were based on circulatory disturbances (CD) which includes hyperemia, haemorrhage, vacuolation. Regressive change (RC) which includes necrosis and progressive change (PC). Results showed that tissue samples of liver and kidney showed an almost equal prevalence in histological alterations in OGB as compared to ARAC, with the liver 20% for both sites and kidney 19.99% against 20%. The Histological Alteration Index, that is, mean fish Index (FI) for OGB is 14.7, making it slightly greater than ARAC, 14.6. It was concluded therefore that the OGB fish pond was slightly polluted.
Keywords: Ecotoxicology, Bio-monitoring, Bio-indicator, Contaminant, Histology, Histopathology.

INTRODUCTION

Fish farming is considered one of the lucrative farming businesses especially in Nigeria. Recently, there has been a geometric increase in the number of people participating in fish farming which is beginning to raise some questions about quality of the facility they use for farming. Fishes are considered a very good source of protein [1].

It was observed, according to Olaleye *et al*., [2] that of over 30,000 MT of various freshwater and brackish water fish species caught in the year 2000, catfishes were more abundant next to tilapias record revealed that the 46,206MT of catfishes were produced in the year 2007. These were consumed locally. With the present population of over 170 million, a projected increase at an annual growth rate of 3.2% and the expected increase in fish demand.

Study area

Experimental Area (Ogbogoro commercial fish pond). Ogbogoro is a community, located in Obior Akpor Local Government Area of Rivers state, Nigeria. It is bounded by, Choba, Rumuekini, Emohua and Diobu. The geographical coordinates are, 4° 50¹ 48¹¹North, 6° 55¹ 50¹¹ East in DMS- Degrees Minutes Seconds or 4.8451°N, 6.9290°E, (in decimal degrees) [3].

Study area environment: the pond is sited in a swampy area which is prone to flood during the mid-raining season. It is located far from residential building. Aside the fishing section in the establishment, there are sections for piggery and poultry farming, although no animals were in them as of the time of my harvest of fishes for my experiment.

The pond is divided into units according to fig. 1.2. Each of this units has nothing less than 200 fishes in them. The fishes in each unit varies in size/age ranging from, hatchling, fingerling, nursery, table size.

Water regulation: According to koi food, the ideal regime for water changes is 10% per week or 20% per two weeks or 50% every six weeks. The percentage of water changed depends on the level of haze or cloudy appearance seen on the water, which is sign of high organics [3] 75% - 80% water must be changed at least

twice per year. In ogbogoro, the pond water is changed every two weeks, completely, although varies depending on the level of pollution of the water.

It has been noted that commercial fish feeds have a high level of concentration, and if eaten in vast quantity will pass through the fish only partly digested and constitute nuisance to the water body and pollution as well. If feeding as a supplement to natural foods in the pond, two or three times a week will be ample. It is advisable that if there is excess food not consumed, it should be netted out to avoid pollution and a reduced quantity be given the next time. Ogbogoro commercial fish pond, feed their fishes during the day with coppens (fish feeds).

Reference Area (ARAC)

African Regional Aquaculture Center (ARAC), was chosen as the control site. It is situated at the training center, Omuihuechi, Aluu in Ikwere Local Government Area of Rivers State. Most of the activities in the center includes research, training, and development of sustainable aquaculture options, in sub Saharan African. It covers an area of 81 hectares of land.

It's a centre of excellence that focuses on multidisciplinary approach to user-driven aqua cultural research, development and training in sub-Saharan Africa geared towards sustainable fish production in the region.

ARAC is affiliated to Rivers State University (RSU) for the award of masters of Science (M.Sc.) and post graduate diploma (PGD) in aquaculture.

Fig-1: Clarias gariepinus

African catfish (Clarias gariepinus) is one of the most important primary treatment for tropical cultured fish due to high growth rate, high stocking-density capacities, and high resistance to poor water quality and oxygen and considered as a model for Eco toxicological studies [4].

Natural Distribution

They are found throughout Africa and the Middle East, and live in freshwater lakes, rivers, and swamps, as well as human-made habitats, such as oxidation ponds or even urban sewage systems. The African sharp tooth catfish was introduced all over the world in the early 1980s for aquaculture purposes, so is found in countries far outside its natural habitat, such as Brazil, Vietnam, Indonesia, and India.

Habitat

It is a nocturnal fish like many catfish. It feeds on living, as well as dead, animal matter. Because of its wide mouth, it is able to swallow relatively large prey whole. It has been known to take large water birds such as the common moorhen. It is also able to crawl on dry ground to escape drying pools. Further, it is able to survive in shallow mud for long periods of time, between rainy seasons. African catfish sometimes produce loud croaking sounds, not unlike the voice of the crow [5].

Natural spawning

Spawning mostly takes place at night in the shallow, inundated areas of the rivers, lakes and streams. Courtship is preceded by highly aggressive encounters between males. Courtship and mating takes place in shallow waters between isolated pairs of males and females. The male lies in a U-shape curved around the head of the female, held for several seconds. A batch of malt and eggs is released followed by a vigorous swish of the female's tail to distribute the eggs over a wide area. The pair usually rests after mating (from seconds up to several minutes) and then resumes mating [6].

There have been works on ecotoxicology with emphasis on Contamination, pollutants, bio-markers and stressors [6-24].

Statement of the problem

High proliferation of fish farm business in Nigeria with inadequate knowledge of sources and breeding pattern and Inadequate or non-regulation of the fish farm business in Nigeria for protection of public health.

Aim

This study was aimed at determining the impact of the commercial fish pond in Ogbogoro, on the health of the cultivated fish.

Objectives

To determine qualitative histological analysis of the liver and kidney cells, the semi-quantitative histological analysis of target organs and the pollution status of the fish pond.

MATERIALS AND METHODS

Phase One (Preliminary Study)

The experimental site was inspected and vital questions were asked as: the number of fishes contained in the pond, type and frequency of fish feed used, treatment administered to fish in poor health condition, mode and frequency of changing the water content of the pond.

A sample fish was harvested and taken to the African Regional Aquaculture Centre for identification by a taxonomist.

Phase Two (Fish Sampling)

Fish Sampling

According to Institute of Veterinary Research and Food Security, Tirana, Albania, The European standards for fish sampling in lakes determined the sampling protocols and methodology developed in the course of fish and fishery monitoring for Prespa lakes. The sampling procedure was based on stratified random sampling.

Control

Control fishes were harvested. This was done by first collecting some water content of the pond into a plastic container which would contain the fishes from the control site to the laboratory. The essence was so that the original aquatic habitat of the fishes will remain the same after harvesting as it was before. Failure to do this will lead to alteration of the fish habitat and questionability of the results which will be gotten. Next, the remaining water content of the pond was drained, then with the aid of a seine; ten table-sized cat fishes were harvested and put into the plastic container in which there was exactly the same water content of the pond.

Study Site

Experimental fishes were harvested. This was done by first getting a good quantity of water from the pond into a well-aerated plastic container designed for the purpose of transporting the fishes to the laboratory. The water content of the pond was drained in order to make the fishes more accessible for a good harvest. Twenty table-sized cat fishes were harvested from the pond

Harvesting of catfish

According to the United Nation's Food and Agricultural Organization, the following are the steps involve in the harvesting of catfish.

The water in the pond was drained to concentrate the catfish, the right catfish seine was chosen and loaded to a seine reel, immersed in the deep end of the pond, and the seine was drawn the pond's harvesting area. The harvest was done at random and seining stopped when the catfish seine was full. The process was repeated till the required sample of size was caught. The sample size was confirmed by measuring on a weighing scale and transported to the laboratory.

Preparation of the Sample

According to American Veterinary Medical Association (AVMA), an acceptable method of euthanasia renders an animal unconscious and insensitive to pain and distress as quickly as possible, followed by cessation of all respiratory and circulatory functions and brain activity.

In line with AVMA guidelines the fishes were sacrificed through cervical dislocation method (severing the spinal cord anterior to the dorsal fin). Then the fishes were surgically opened on the ventral side of the fish for excision of the organs.

Histological Assessment

About 20 table-sized fish were harvested from Ogbogoro commercial fish pond and 10 table sized fish were also harvested from ARAC fish pond and organs of interest (gill, liver, and kidney) were extracted for histological assessment. This assessment involves the microscopic study of the tissues and is divided into two qualitative and semi quantitative analysis.

Qualitative Analysis

Tissue Processing

This assessment involves histological processes in order to view the cells of harvested organs with the aid of a light microscope, these processes are as follows:

Step 1 (Pithing)

This is done by positioning the pointer end of a knife above its brain. Push it down quickly into the brain cavity of the fish.

Locate the spot marked on the head, cut through with a sharp knife.

Step 2 (Dissection)

Cut a slit along the belly of the catfish all the way to the anal fin. This is done with the aid of a dissecting kit (i.e., a scalpel and a scissors to be precise) to harvest the organs of interest from the sample fish while the heart of the fish is still beating.

Step 3 (Fixation)

This is done immediately after the organ of interest has been harvested from the sample fish, it involves transferring the harvested organ into a specimen bottle containing a fixative in this case 10% formal saline was used as the fixative. Fixatives aid in the preserving the tissue architecture of the organ and prevent putrefaction.

Step 4 (Dehydration)

This is the removal of water molecule from the fixed tissue with graded alcohol and aid the penetration of the clearing agent since it is not miscible with water. Usually harvested organ is passed through 50% alcohol, then 70%, 90%, and 100% (absolute) so as to remove the water molecule gradually and steadily but in this study the fish organs were place in 70%, 90%, 95% and 100% for 6 hours in order for the organs to dehydrate well, this is due to the fact since they are fishes they live in water there will be large amount of water quantity in their organs.

Step 5 (Clearing)

Here, a clearing agent in this case xylene is used to remove the alcohol in order to aid the penetration of paraffin wax since paraffin wax is immiscible with alcohol. The dehydrated tissue is the placed in a glass specimen bottle containing the clearing agent (xylene).

Step 6 (Impregnation/Embedding)

This involves placing the tissue cassette and place in melted wax in order for the paraffin wax to replace the water molecules and make the tissue hard for sectioning after which the tissue is placed in mould containing molten wax and allowed to solidify in the paraffin wax thereby forming a tissue block. The tissue block is then cast to a wooden lock in order to make it easy to be placed in a microtome for sectioning.

Step 7 (Sectioning)

This used to produce very thin slice (sections) of the tissue block in ribbons (which would be viewed under a light microscope) with the aid of a microtome. Sectioning makes it easy for tissue to be stained.

Step 8 (Staining)

This involves rinsing the thin tissue section to water through xylene, graded alcohol and then distilled water (i.e. 100%, 90%, 70%, and 50%) and then the section is stained with the H & E stain which is used to view the tissue component.

Step 9 (Coverslipping)

This involves using a cover slip to cover the tissue section on the slide, and then it is mounted for viewing with the aid of a light microscope.

Semi Quantitative Histological Analysis

A qualitative assessment protocol was used to quantify histopathological alterations observed in the sections of each of the organ. A qualitative histopathological assessment was done using CX1 Olympus light microscope. Tissue sections were scanned on 400x magnification. The result were semi-quantitatively assessed using part of a scoring system. Bernet *et al.*, 1999 modified from the protocol by [29]. In brief, the tissue samples were assessed by identifying histopathological alteration in terms of reaction patterns including:

1. Circulatory disturbance
2. Regressive changes
3. Progressive changes
4. Inflammatory responses

Neoplasia; if identified, the alteration was given an importance factor which represents the potential of the alteration to affect fish health: 1 (alteration is reversible; 2 (alteration is reversible if the stressor is neutralised); 3 (alteration is irreversible). A score value, representing the occurrence of the alteration throughout the tissue was also assigned: 0 (absent), 2 (mild), 4 (moderate), and 6 (severe). The score value and the importance factor for each alteration were multiplied and these results for all the alterations identified in a single organ were then summed to give an organ index per fish. Thus, 2 organ indices were calculated: Liver index; Kidney index. These organ indices were calculated for each sample fish. A mean of each organ index was calculated for each sample group (experimental and control group) and was used to compare the same organs between the groups.

The sum of the two indices per fish yielded a total fish index value. This index indicates the combined histological response of the sampled organs for the individual fish. A mean fish index was calculated for the total sample group per species.

Mathematical calculations of lesion indices

Where org= organ; rp= reaction pattern (constant); alt= alterations; a= score value; w = importance factor.

1. Organ index: The organ index (Iorg) represents the extent of damage to an organ. It allows for comparison of the extent of damage of the same organ in different individuals and is calculated as follows:

   Iorg= $\sum T \sum alt$ (aorg rp alt x worg rp alt).

2. Total fish index: The fish index (Ifish) signifies a measure of the overall health status based on the lesions observed. It is also possible to compare individuals as the Ifish for each fish is calculated the same way:
Ifish =ΣorgΣrp Σalt (aorg rp alt x worg rp alt).

Thermore, a modified classification system by [29] based on a scoring scheme by Zimmerli *et al.*, [31] was employed to evaluate the degree of histological changes. This classification system is based on the calculated mean organ index values.
Class 1 (index value <10): Slight histological alterations.
Class 2 (index value 10-25): Moderate histological alterations.
Class 3 (index value 26-35): Pronounced alterations of organ tissue.
Class 4 (index value >35): Severe alterations of organ tissue.

Statistical Analysis

One way anova statistical method was used to analyse the organ indices of the specimens from Ogbogoro and ARAC at a significant level of 0.005

RESULTS AND DISSCUSION

Table-1: The percentage prevalence of Liver histopathology of fishes harvested from OGBOGORO and ARAC

ALTERATION	PREVALENCE (%)	
	OGBOGORO (n=20)	ARAC (n=10)
Circulatory Disturbance (CD)		
Haemorrhage	25	20.75
Vacuolation	46.25	37.74
Regressive Change (RC)		
Necrosis	11.25	15.09
Foci of Cellular Alteration		
Vacuolated Foci	7.5	62.5
Necrotic Foci	10	16.98
Average % Prevalence	**20**	**20**

Table-2: Statistical Analysis using One Way Anova

Gill	N	Mean	Standard Deviation	F-test	Significance
1.00	10	11.600	11.462	0.656	0.425
2.00	20	14.600	8.512		
Total	30	13.600	9.503		

The analysis shows that there is no significant difference between the two data (P>0.05)

Liver Histopathology

A variety of histological alterations were identified in the liver tissue (Figure-2a-e). These alterations include vacuolated hepatocytes, necrosis, haemorrhage, foci of cellular alteration (FCA) which are vaculated and necrotic foci. Vacuolated hepatocytes and haemorrhage were more prominent in fish specimen from Ogbogoro. Percentage prevalence of FCA and RC were not so different between Arac and Ogbogoro (Table-1).

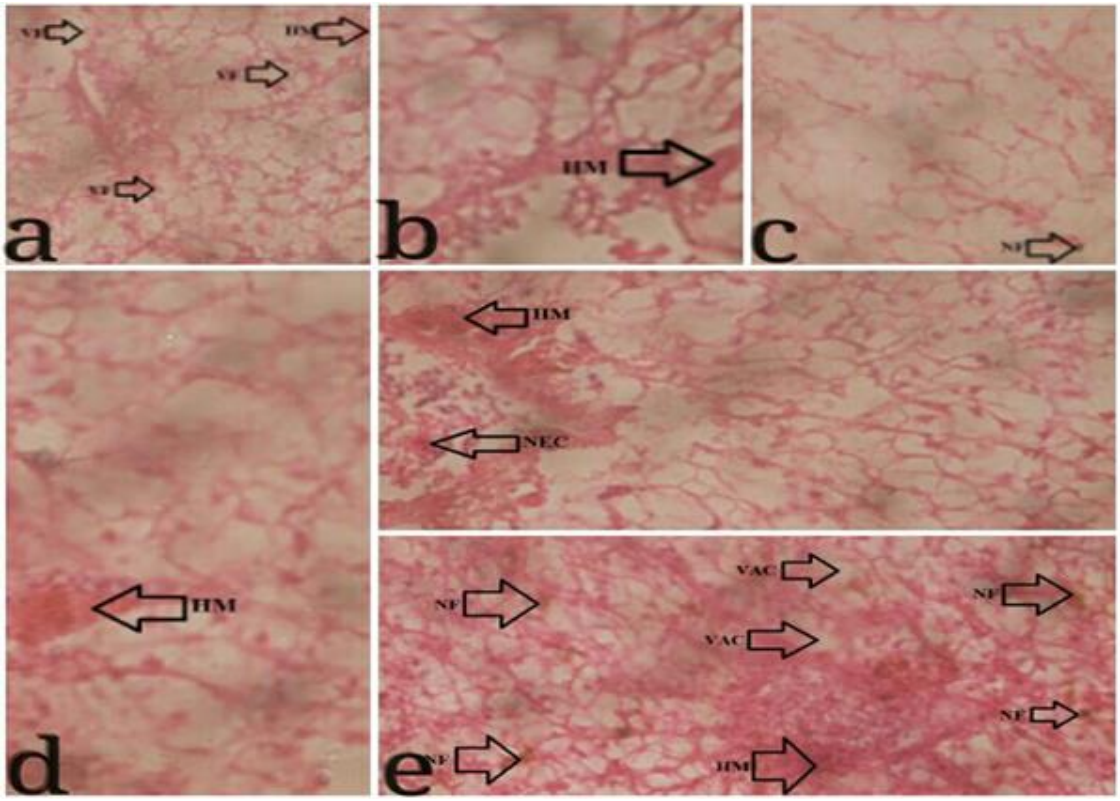

Fig-2: (a-e). Liver microscopic structure {H and E, x400}; a) Control showing hemorrhage(HM) and vacuolation; b) Haemorrahge (HM); c) Necrotic Foci (NF); d) and e). vacuolation (vac), hemorrhage and necrotic foci

Table-3: The percentage prevalence of Kidney Histopathology of fishes harvested from OGBOGORO and ARAC

ALTERATION	PREVALENCE (%)	
	OGBOGORO (n=20)	ARAC (n=10)
Circulatory Disturbance (CD)		
Haemorrhage	8.77	0
Vacuolation	19.29	14.29
Regressive Change (RC)		
Necrosis	15.79	19.05
Foci of Cellular Alteration		
Structural Alteration	29.82	28.57
Melano-Macrophage Centers	26.32	38.09
Average % Prevalence	**20**	**20**

Table-4: Statistical Analysis using One Way Anova

Gill	N	Mean	Standard Deviation	F-test	Significance
1.00	11	14.909	8.263	1.721	0.200
2.00	20	11.100	7.440		
Total	31	12.451	7.826		

The analysis shows that there is no significant difference between the two data (P>0.05)

Kidney Histopathology

Histological alterations noted in the kidney tissue were only circulatory disturbances (CD) regressive changes (RC). These alterations included vacoulation, structural alterations and MMCs (Figure-3).

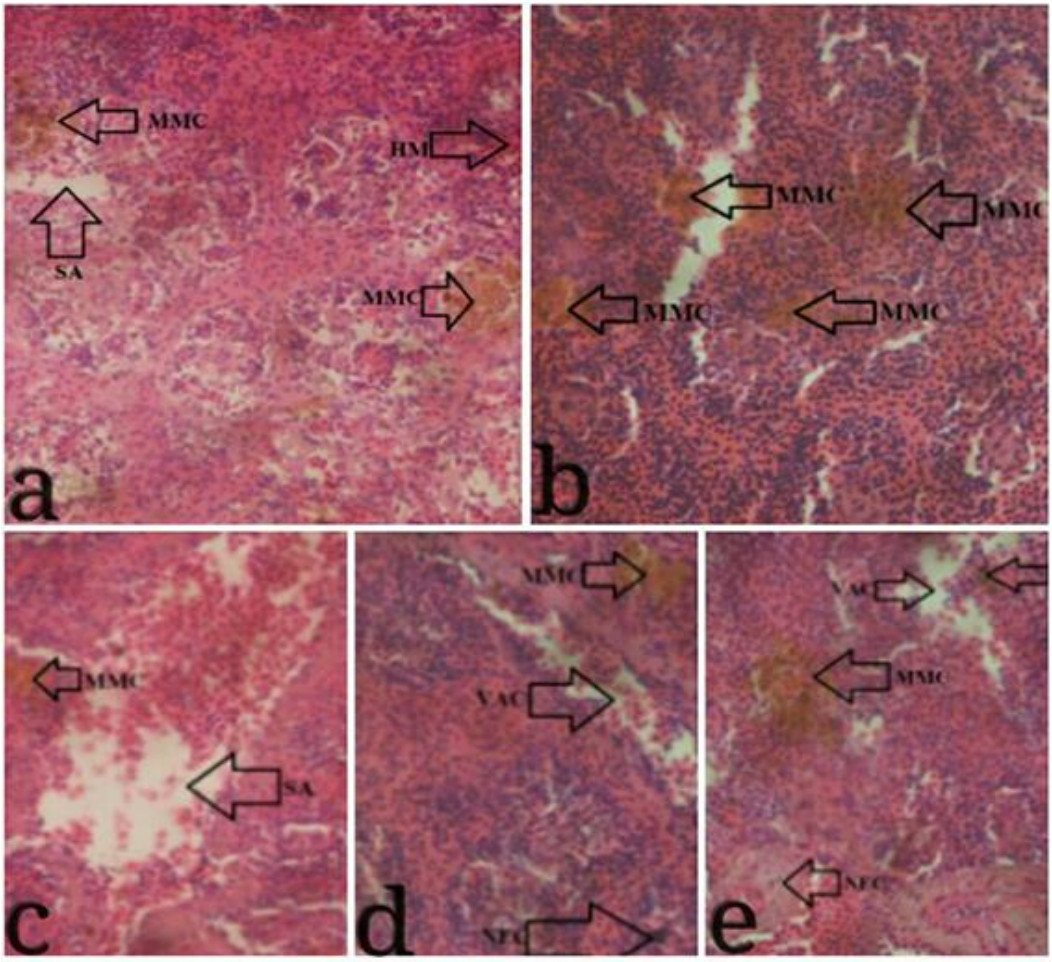

Fig-3: (a-e). kidney microscopic structure {H and E, x400}; a) Showing hemorrhage, melanomacrophage centers(MMC); b) Showing MMCs; c) Showing structural alterations(SA); d) Showing necrosis(NEC) and lipid vacuolations(Vac); e) Showing MMCs, VAC, and NEC

DISCUSSION

Liver Histopathology

Histological alterations observed were structural alterations such as hypertrophy; circulatory disturbance such as vacuolation, necrosis of hepatic tissue and haemorrhage.

The liver is a detoxification organ and is essential for both the metabolism and the excretion of toxic substances in the body [25].

In the current study circulatory disturbances, regressive changes, and focal cellular alterations were identified. The histological responses in the liver were mostly associated with circulatory disturbances and regressive changes including vacoulation, necrosis, increase in FCA.

Focal cellular alteration was also identified in both fish species in the study [26] has previously reported the occurrence of this alteration.

Kidney Histopathology

The kidney receives the largest proportion of post branchial blood and therefore renal alterations might be good indicators of environmental pollution [27, 28]. In the current study histological alterations in the kidney were identified. These alterations are mostly associated with progressive and regressive changes, which may lead to changes in blood pressure passing through the kidneys and thus affecting the kidney function.

These were mainly regressive changes and included, necrosis, degeneration as well as increase in MMCs.

Necrotic changes have been observed in fish kidney exposed to various chemicals by various authors [27, 28, 29]. Alterations such as necrosis may lead to functional problems ultimately leading to the death of fish.

Increase in MMCs was also observed in some fish specimen in the current study [26] noted the presence of macrophages in exposed to heavy metals [26]. Also obtained similar results to that of the current study in *C. gariepinus* from a polluted site.

Melanomacrophage centers were a common occurrence in the kidneys of both species. It has been suggested that MMCs are a normal characteristic in fish tissue but an increase in the number or size of these structures can be as a result of a number of factors, including toxicant exposure [29, 30] and possibly age.

CONCLUSION

The level of the alterations in the liver and kidney cells indicate that Ogbogoro commercial fish farm is slightly polluted and could be categorized as second class of level of alterations according to Zimmerli's classification.

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