

# Liquid-Crystal in Embryogenesis and Pathogenesis of Human Diseases

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## 1. Introduction

In 1979, a systematic publication summarizing the state of research on liquid-crystals in biological organisms was published [Brown GH *et al* 1979]. After this historic publication on liquid-crystals and biology, the field remained largely dormant for more than two decades. However in 1978 and 1979, Haiping He and Xizai Wu, who had continued pursuing this field despite international disinterest, reported their findings on liquid-crystal involvement during chicken development. For the first time, they revealed that massive quantities of liquid-crystals in the liver, yolk sac, blood, and many other developing tissues and organs of chicken during embryogenesis. Their later studies also reported similar liquid-crystalline structures during fish development. In 1988, another group reported the existence of vaterite CaCO<sub>3</sub> within the liquid-crystals found in yolk fluid, identifying the spherical calcified structures first reported in 1979 as one of three iso-forms of calcium carbonate [Feher G 1979, Li M *et al* 1988]. Subsequent studies have identified liquid-crystalline structures to be omnipresent in the liver during avian development [Xu XH *et al* 1995a, 1995b, 1997]. Recent studies have revealed that liquid-crystals play a critical role in the preservation of calcium and other trace elements required for embryo development [Xu MM *et al* 2009, 2010, 2011; Xu XH *et al* 2009, 2011a].

In recent years, more and more human diseases have been related to liquid-crystals. Amongst these diseases are genetic disorders, such as Age-related Macular Degeneration [Haimovici R *et al* 2001], steatohepatitis and atherosclerosis [Goldstein JL *et al* 2008], and Anderson-Fabry Disease [Xu MM *et al* 2009]. For Fabry patients, the accumulation of liquid-crystal or concentric lamellar bodies glycosphingolipids in neurons can cause severe neuroradiological abnormalities, including periventricular white-matter signal intensity abnormality and single/multiple lacunar infarction, large ischaemic cerebral infarct and

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posterior thalamic involvement [Ginsberg L *et al* 2006; Lidove O *et al* 2006; Moore DF *et al* 2003]. This accumulation also occurs in the cardiac vascular system, resulting in angina and varied complications, ranging from arrhythmia to myocardial infarction and heart failure [Pironi M *et al* 2006; Linhart A *et al* 2007]. Although a large volume of publications indicate an irreplaceable role for liquid-crystals in both normal physiological development and pathogenesis, the exact function of liquid-crystal is uncertain.

In this chapter, we will summarize current research around liquid-crystal involvement in embryogenesis and how these normal embryonic events, when triggered at inappropriate times, can lead to pathogenic events. Current publications indicate that liquid-crystals are often involved in normal embryogenesis, but the appearance of liquid-crystals in post-natal development often heralds pathogenesis in mature tissue. Normally embryogenic events can be triggered by a variety of factors, such as genetic predispositions or bacterial infection. As part of normal embryogenesis, the formation of liquid-crystals indicates erroneous initiation of normal growth, often leading to disease.

## **2. Methodology for liquid-crystal function in medical biology and embryogenesis**

Liquid crystal refers to a material's physical state. As the term suggests, liquid-crystals display both liquid and solid tendencies. This fluid characteristic perfectly matches the properties of a living organism. However, this same adaptability to life introduces some critical attention to experimental approaches when studying liquid-crystals *in vitro*. In this section, we will focus on research approaches used for studying liquid-crystals in biological embryogenesis and human pathogenesis. The preeminent combination of all procedures could perform excellent inspection on the liquid crystal [Xu XH *et al* 2011a].

### **2.1 Animal sample collection according to animal IACUC**

In the United States, any research application involving laboratory animals is required to be approved by the Institutional Animal Care and Use Committee (IACUC) prior to experimentation, if the research is funded by a federal agency such as the National Institute of Health. All animal procedures must also follow guidelines approved by the home institution's Animal Care and Use department. For mouse tissue and organ harvest, mice must first be euthanized via CO<sub>2</sub> asphyxiation followed by cervical dislocation. After the necessary tissues have been harvested, the mouse remains should be sealed within plastic bagging and frozen for temporal storage in the animal core facility. Permanent treatment of animal body will be carried out by the core.

In most instances of commercial mice, such as 129SvEv and DBA/2J breeds from the Jackson Laboratory (Bar Harbor, ME), Harland Laboratories (Indianapolis, IN), and other commercial sources were bred under standard conditions and sacrificed by cervical dislocation according to IACUC regulation. All tissues were harvested immediately and snap frozen with liquid nitrogen upon dissection. For timing experiments using mouse embryo, appearance of the vaginal plug was designated as embryonic day (E) 0.5, and embryos were either collected from the stage of interest, or pregnancies were allowed to reach term. For postnatal studies, newborn mice were collected at the day of birth (postnatal day, P).

For avian experiments, fertilized chick eggs such as White Leghorn (*Gallus domesticus* L.) are normally incubated at 37°C with relative air humidity of 60%. Experiments are conducted and samples harvested at time-points according to experimental procedure. The age of embryos is documented as day of incubation (D) and the postnatal age of chicks is documented as Postnatal (P). Collections of tissues and organs are the same as described above and can be found within references [Xu MM *et al* 2009, 2010, 2011; Xu XH *et al* 2009, 2010, 2011ab].

## 2.2 Cryo-section histology analysis

For purely biological studies of embryogenesis, both paraffin section and cry-section are good options for well-preserved tissue and organ samples. However, for liquid-crystal functional studies during embryo development and pathological events, cryo- or frozen-section are preferred over paraffin sections, as the cryo/frozen methods preserve more of the sample's original characteristics than the more chemically intensive paraffin preservation method. If possible, fresh sample smear-slides are also preferred. [Xu MM *et al* 2009, Xu XH *et al* 2009].

Though the previous methods allow for long term storage, fresh smear-slide preparation arguably retains the most fidelity to *in vivo* systems as the tissue is not processed in any way prior to observation. In smear-slide preparation, samples are harvest from the embryos at different stages. Each sample is then immediately smeared on a slide wetted with PBS (PH 7.4) buffer then mounted with a cover slip. This method is best observed by polarization microscopy, which can be conducted immediately following the sample smear preparations.

When in need of a method for long term storage or if retaining the physical structure of the tissue is desired, sryosection preparation is preferred. Embryo samples are submerged in the cryomatrix embedding agent (OCT) and placed in an aluminum foil basket or other suitable container to be dipped. The samples are then frozen by dipping the foil basket or the container into liquid nitrogen. The now frozen tissues embedded in the OCT block can now be placed on the cryostat microtome and sections cut for experimentation. Thicker cuts 10~30 mm are preferred for polarization microscopy and thinner cuts of ~5mm are preferred for H&E staining. The samples collected using these two procedures should be mounted with 20% of glycerol in PBS (PH 7.2) and sealed before proceeding to further analysis.

## 2.3 Immunohistochemistry and confocal microscopy

After collection and smear-section preparation, samples should be washed with PBS, fixed in 4% formaldehyde in phosphate-buffered saline (PBS), and permeabilized for 10 min in PBS containing 0.25% Triton X-100. Immunocytochemical staining can be perform performed by incubating the samples with primary antibodies in PBS/Tween (PBS containing 0.1% Tween 20 and 3% BSA) for 1-2 hr, followed by incubation with appropriate secondary antibodies diluted according to the manufacturer's recommendation in PBS/Tween solution for 1-2 hr. Stained samples can then be visualized with confocal microscope.

Immunohistochemistry is a powerful approach to unveil distribution of protein of interest, which can be used to locate liquid-crystal related proteins in tissues and organs. However,

because this method requires the use of damaging solutions such as formaldehyde, Triton X-100, and Tween containing blocking buffer, results should be compared to smear-samples for confirmation of signal localization corresponding to liquid crystal.

## 2.4 Polarization microscopy and thermal phase-transition

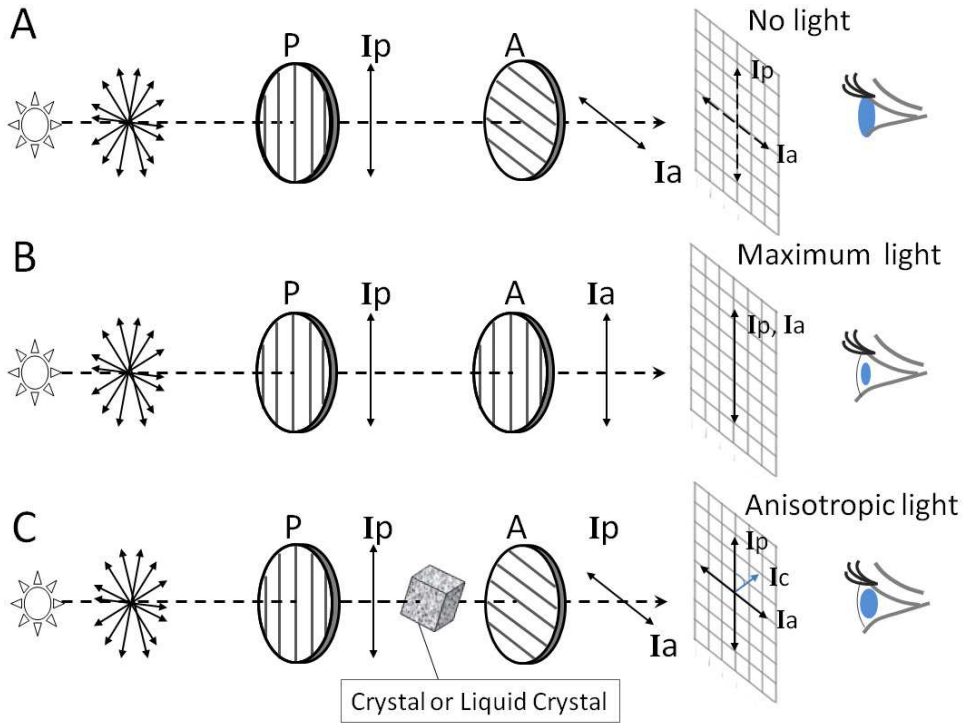
Polarization or polarized microscopy is an irreplaceable tool for studying materials with refractory activity. In the case of liquid crystal study, two states of material, crystal and liquid crystal, have birefringent activity. In our studies, we have found microscopy to be an invaluable tool in examining the light activity of liquid crystals in animal tissue.

Natural light such as light from an ordinary light source is called non-polarized because it vibrates in random directions. Polarized light on the other hand, travels within a single plane and presents with vertical vibrations that can produce linear, circular, and elliptical polarized light. A polarizing plate or polarizing prism is often used as a polarizing filter to remove all but one wave with the same directional vibration.

Observing liquid-crystals under polarized light requires a basic understanding of light polarization. To create a polarized light, two devices, the primary and secondary polarizing devices, are oriented perpendicular to each other as crossed nicols to filter polarized light from normal lighting. The primary device will filter a polarized light from the light source, while the secondary device cuts the light depending on orientation of the two devices. These primary polarizing device and secondary polarizing device are called Polarizer (P) and Analyzer (A), respectively. In perpendicular nicols, the analyzer is rotated to be perpendicular to the polarizer. Since both nicols act as filters, the analyzer cancels out the polarized light from the polarizing lense, to yield no light to the observer (Figure 1A). In parallel nicols, the analyzer is rotated so that the direction of the transmitting polarized light is parallel with the polarizer. This allows polarized light transmitted via the polarizer to travel through the Analyser, maximizing the amount of light transmittance (Figure 1B).

The light bending ability of liquid crystals can be thought of as an additional nicol. When polarized light launching through a crystal or liquid crystal materials is divided into two linearly polarized light rays, these two rays possess mutually crossing vibration directions, called birefringence (double refraction). A crystal or liquid crystal that refracts in this way is called anisotropy. When an anisotropic crystal or liquid crystal is inserted between a polarizer and an analyzer in a crossed nicols state, the crystal or liquid crystal changes the state of the polarized light and the light to pass through partially (Figure 1C). These changes are different depending on various crystal or liquid crystal, which can be utilized to determine characteristics of an anisotropic material, liquid crystal in this case.

Using this light-bending property of liquid crystals, thermal-probe sample stage in conjunction with polarization microscope, can be used to monitor and record phase transition of liquid crystals. When a sample is observed with crossed-nicols, the anisotropic texture of crystal or liquid crystal will vanish once the temperature of stage reaches the point of phase transition from liquid crystal to isotropic states. This technique is an effective method for observing the liquid crystal properties of biological samples containing liquid crystals [Xu MM *et al* 2010, 2011; Xu XH *et al* 2011a].



A. Crossed nicols;  
 B. Parallel nicols;  
 C. Anisotropy between crossed nicols.  
 P: polarizer  
 A: analyzer

Fig. 1. Light path of polarization and anisotropy

## 2.5 Small angle X-ray scattering (SAXS) and X-ray diffraction (XRD)

When X-ray beam of a particular wavelength diffracts from atoms in a crystalline structure, the wavelength of the x-ray ( $\lambda$ ), scattering angle ( $\theta$ ), integer representing the order of the diffraction peak ( $n$ ), and inter-plane distance ( $d$ ), usually the distance between atoms, ions, molecules, follow the Bragg's Law (Figure 2A).

$$2d \sin \theta = n\lambda$$

This equation predicts that different layers of atoms in lattice planes will generate various distances corresponding to peaks. Crystal samples, multiple peaks will be present in a widespread diffraction angle ( $2\theta$ , XRD; Figure 2B), while liquid crystal exhibit fewer peaks within an area of small scattering angles ( $2\theta$ , SAXS; Figure 2C). For crystals or liquid-crystals within biological samples, once liquid-crystals or crystals have been isolated or extracted, temperature and other conditions must be tightly controlled to retain the original characteristics and diffraction pattern of samples.

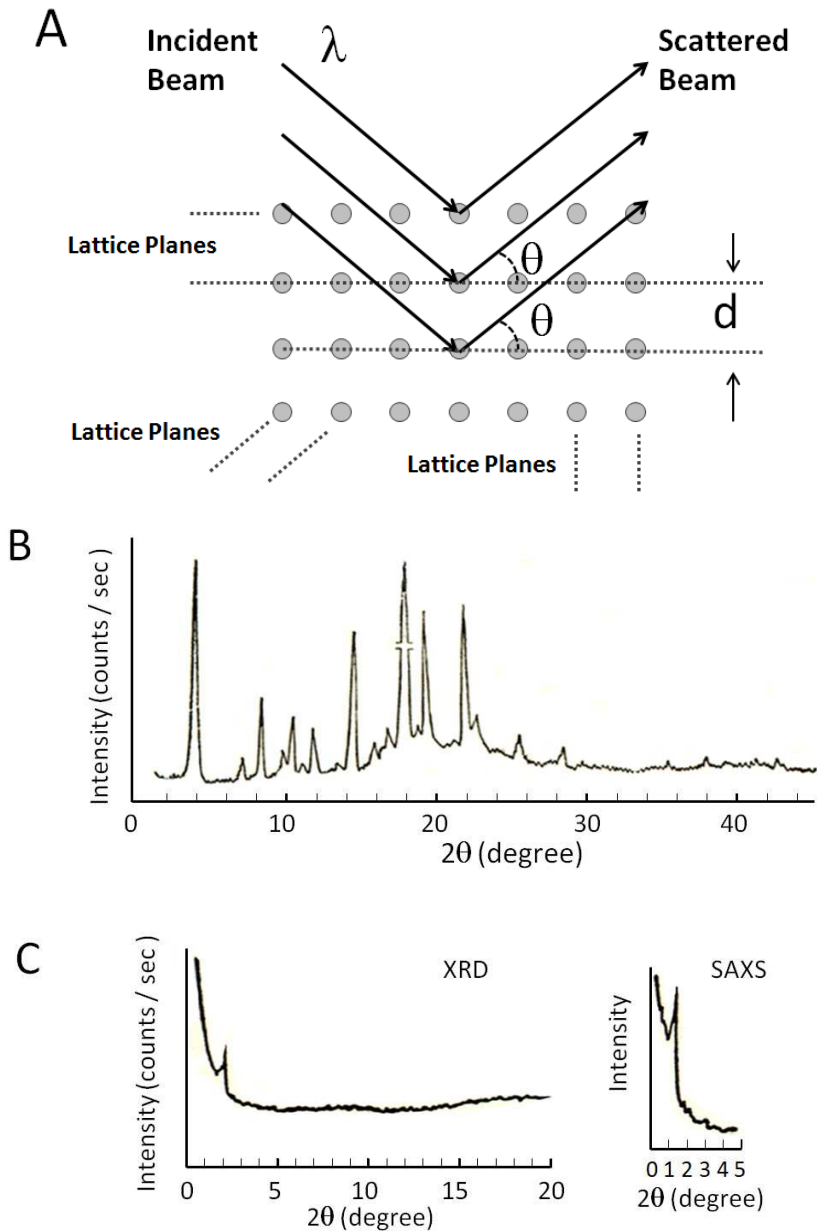


Fig. 2. Bragg's law and the diffraction patterns of crystal and liquid crystal. Relationship between wavelength, scattering angle, and distance of lattice planes (A). XRD pattern of crystal cholesteryl oleate within 50 degree of diffraction angle ( $2\theta$ ) (B), and SAXS pattern of liquid crystal in embryonic liver within 50 degree ( $2\theta$ ) (left in C) and within 5 degree of scattering angle degree ( $2\theta$ ) (right in C).

## 2.6 Generation of mouse model using daily diet procedure

Mice are obtained from Jackson Laboratories or another commercial facility. Male or female pups are split into two groups. Group one is fed on the standard chow diet (Harlan Teklad #2018 rodent chow) with 48% carbohydrate, 16% protein and 4% fat. Group two is on a low carbohydrate high protein diet with 15% carbohydrate, 58% protein and 26% fat, which we was designed to mimic the typical Western diet.

## 3. Comparative summary of liquid-crystals in embryonic tissue development and post-natal pathology

As discussed earlier in the chapter, embryonic tissues and organs of several animal models have exhibited traces of liquid-crystals during embryonic development stage that did not persist postnatally. In liver and yolk sac, massive liquid-crystals are present from embryogenesis to early post-natal development. In this section, we will summarize the characteristics of liquid-crystals in different tissues in comparison to the liquid-crystals found in human diseases (Table 1).

### 3.1 General characteristics of embryonic liquid-crystal

During embryogenesis, liquid crystals are widely distributed in the tissues of vertebrates and invertebrates, including *Apis cerana chrysalis*, fish, reptile, avian and mammal early embryo in vitro [X XH *et al* 1993, 2009, 2011a, Xu MM *et al* 2009 2011]. In chicken development, more than twenty different organs and tissues exhibit liquid crystal droplets including liver, meso- and metanephros, lungs, blood in heart, and brain. The presence of liquid crystal normally appears at different developmental stages depending on the tissue type, and lasts until early postnatal stages. The earliest liquid crystal droplets appear on the inner embryonic disc during the second day of development [He H *et al* 1978]. Regardless of their distribution, however, the liquid crystal droplets eventually vanish within three to four weeks into the postnatal period, also depending on tissue type maturation [X XH *et al* 2009, 2011a].

During chicken development, two particular organs, the liver and yolk sac, exhibit massive birefringent liquid crystal at higher levels than all over tissues in the developing embryo. The hepatic birefringent particles are mainly composed of cholesteryl oleate, cholesterol, lecithin and an unidentified component [Xu XH *et al* 1992, 1995a, 2011a]. These liquid crystal droplets are situated in hepatocytes of the hepatic cord region. In the kidney development, LC droplets can exist in the cytoplasm of epithelial cells and the lumen of proximal tubules in the mesonephros and metanephros. The existence of LC in two very different organs indicates that the liquid crystal likely plays many different roles during the development or a similar role in many tissues.

### 3.2 Decrease-rate dependent thermal phase transition

Under polarization microscope, liquid crystal exhibit Maltese-crosses optical textures, while crystals produced more angular (needle-like, rhombus, or dot-shape) diffraction patterns. The two states also reacted differently to pressure experiments, with the liquid-crystals dividing into smaller Maltese-cross droplets, while crystals fractured under duress [Xu XH *et al* 2009, 2011a].

Thermal phase transitions have also been revealed in liquid crystal obtained from various tissues (Table 1). Not surprisingly, with thermal stage temperature increase, the birefringent liquid crystal droplets transit to non-refracting isotropic droplets. With temperature decrease, the liquid crystal droplets transit into crystal. However, when the isotropic droplets cool, two different results, controllable by rate of temperature decrease, were possible. If the rate of temperature drop is fast (the slide is placed on a 4°C plate), then the isotropic droplets will transition into liquid-crystals. However, if the rate of temperature decrease is slow (temperature is allowed to drop in step with the slowly cooling copper thermo-controller) then the isotropic droplets will transit to crystal (Figure 3). This finding

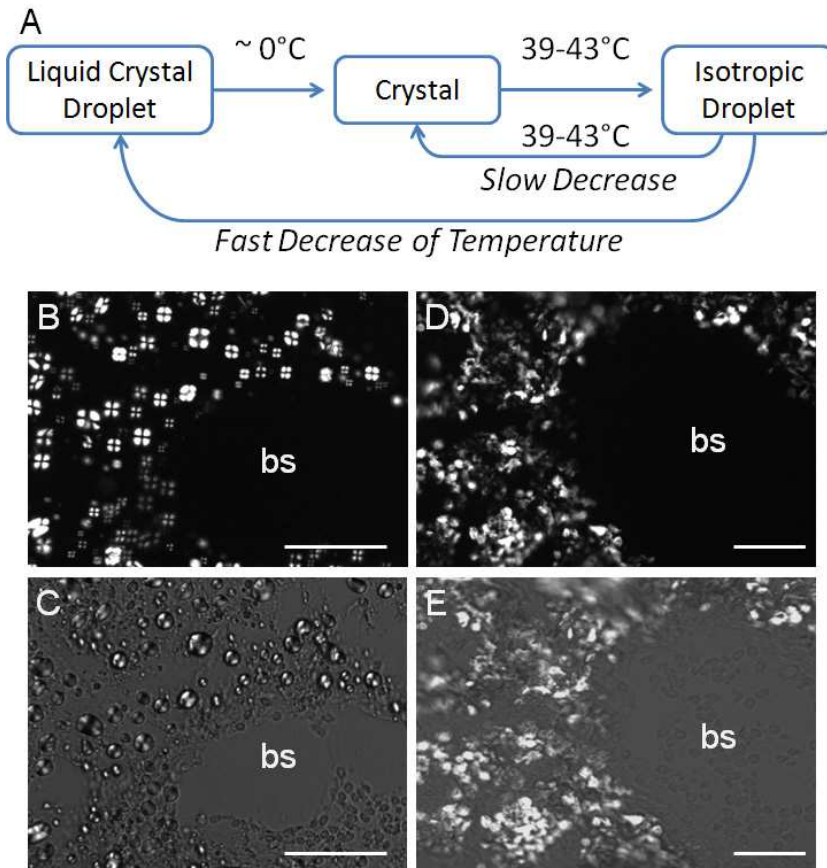


Fig. 3. Thermal phase transitions of liquid crystal droplet, crystal and isotropic droplet. The phase transition from isotropic droplet to crystal or liquid crystal depends on the rate of temperature decrease (A). B and C exhibit the hepatic liquid crystal droplets in crossed nicols with 90 degree of angle (B) and 45 degree (C). D and E show the hepatic crystal in crossed nicols at 90 degree of angle (D) and 45 degree (E), which transitioned from liquid crystals. Anisotropic liquid crystals locate in hepatocytes in the cords and are absent in the blood sinus (bs). Bars, 60  $\mu$ m.



was initially established in embryonic hepatic liquid crystal then [Xu XH *et al* 1995] proven to be a general phenomenon in embryonic liquid crystal of other tissues and organs [Xu XH *et al* 2011 and Table 1].

Embryonic Tissue/Organ*		Temperature of Phase Transition (°C)			Liquid Crystal in Diseases	Ref.
		LC to Isotopic	Crystal to Isotopic	Isotopic to Crystal **		
Liver	E14	37.6 ~41.5	39.4~42.0	35.6~36.3	Steatohepatitis Gaucher disease	12, 13, 40, 43, 44, 51
Kidney Mesonephros	E8	36.6~40.5	38.1~40.8	38.6~40.8	Fabry- Anderson	1, 6, 33, 36
Metanephros	E14	36.2~40.2	37.9~41.1	38.6~40.7	disease	
Lung	E17	37.1~41.3	38.6~41.6	35.7~35.6	Gram-positive bacteria sputum	37
Aorta	E	37.8 ~41.1	NE	NE	Atherosclerotic lesions	10, 11, 19, 20
Vein	E	37.2 ~41.4	NE	NE	Foam cell abnormality	4, 5, 12, 13, 48
Heart	E	36.8 ~40.0	NE	NE	-	15
	P 10	Non- detectable	Non- detectable	Non- detectable		
Eye (retina)	E19	37.2 ~41.1	39.3~41.1	35.4~36.5	ARMD	14, 31, 32,34
	P6	Non- detectable	Non- detectable	Non- detectable		
York sac	E2~3	38.5~42.5	44.3~46.3	46.2~46.6	-	38, 47
	E17~9	38.9~42.3	44.1~46.3	46.3~47.2		
Blood	E9	NE	NE	NE	Gaucher disease	13, 26, 48

\*The data from domestic fowl Taihe

\*\*These temperatures are documented with slow decrease of thermal stage.

NE: Not examined

Table 1. Liquid-crystal characteristics in embryonic tissues/organs and post-natal diseases

### 3.3 SAXS and XRD measurements of liquid-crystal and crystal

SAXS pattern can be documented with massive hepatic liquid crystals. Within 50 degree of  $2\theta$ , only one scattering peak can be detected corresponding to 35 Å in small angle area, indicating the distance of molecular layers of liquid crystal droplets. Using this approach, the period distance of liquid crystal from the fat body of *Apis cerana chrysalis* [Xu XH *et al* 1994a] and hepatic liquid crystals of various avians have been documented [Xu XH *et al*

1995, 2009, 2011]. Crystals transitioned from liquid-crystals can generate many more diffraction peaks corresponding to more crystallographic planes. Oddly, XRD pattern reveals more orders on the hepatic crystals.

SAXS and XRD measurements elucidated significant differences between hepatic liquid crystals obtained from different species of avian. In Taihe fowl, the SAXS scattering of the hepatic liquid crystal expresses a strong peak at 2 $\theta$ . But the peak is weak or absent in its crystal XRD diffraction. In pigeon, the SAXS hepatic liquid crystal peak does have a significant XRD diffraction pattern. This difference indicates that although liquid crystal can be found in the same tissues of the two avians, they likely contain different chemical components (Table 2).

### 3.4 Liquid crystals in human disease

Unlike during embryogenesis, no liquid crystals are found in postnatal development or in normal physiological systems. However, liquid crystalline structures have been reported in different tissues during pathological processes, including atherosclerosis, abnormal lipid depositions, Age-Related Macular Degeneration, and active monocytes.

In 95 patient samples of atherosclerotic lesions, liquid crystals composed of cholesterol, cholesterol ester, and phospholipid were observed [Lang PD *et al* 1970]. This data has been confirmed by another group [Goldstein JL *et al* 1977] and mimicked within *in vitro* systems [Goldstein JL *et al* 1979]. Maltese-crosses, indicating liquid-crystals, have also been found in lipid depositions accumulating in smooth muscle and foam cells [Kruth H 2001]. These liquid crystal depositions in the vascular wall were found to be low density lipoprotein-cholesteryl esters mediated by cell surface receptors [Goldstein JL *et al* 1977, 1979, 1997, 2008; Brown MS *et al* 1974, 1974].

Age-Related Macular Degeneration (AMD) is the leading cause of severe vision loss in adults over 50. The Center for Disease Control and Prevention estimates that 1.8 million people are suffering AMD and over 7 million are at substantial risk for vision loss from AMD in United State. Liquid crystal Maltese's-crosses and crystals structure were observed in the drusen of retina in ARMD patients [Small DM 1970, 1986, 1988; Haimovici R *et al* 2001]. In these patients, drusen are much bigger than normal and are filled with accumulated anisotropic structures.

Recently, cytoplasmic accumulation of liquid-crystal like droplets have also been found in monocytes, macrophages, and squamous epithelial cells of sputum from a patient affected with Gram-Positive Bacteria [MM Xu *et al* 2011]. In sputum collected during the recovery phase of respiratory infection, massive Maltese-crosses were fully loaded in host cells. Though the mechanism of formation for these liquid-crystal like droplets has discovered, further study could lead to new perspectives on post-infection removal of infectious agents.

In addition to Fabry-Anderson Disease [MM Xu *et al* 2009], birefringent particle accumulation are also observed in Gaucher disease [Goodman ZD *et al* 2009, Hillman RS *et al* 2005]. This disease is a lysosomal storage disorder, in which deficiency of glucocerebrosidase causes a buildup of fatty substance glucocerebroside in the monocyte and macrophages of certain organs. As the observations were made on biopsy samples, the

birefringent fragments observed are likely crystals generated from native liquid crystals as an artifact of freezing. This birefringent accumulation was observed in liver and blood of the patients as well (Table 1).

Newborn Chicken				Newborn Pigeon			
Crystal from LC		Liquid Crystal		Crystal from LC		Liquid Crystal	
XRD		SAXS		XRD		SAXS	
I / I <sub>0</sub>	d (Å)	I / I <sub>0</sub>	d (Å)	I / I <sub>0</sub>	d (Å)	I / I <sub>0</sub>	d (Å)
-	-	100	37.0	46	38.10	100	38.02
100	19.55	-	-	32	19.48	-	-
5	17.63	-	-	11	12.10	-	-
1	10.59	-	-	10	10.71	-	-
6	9.44	-	-	30	7.21	-	-
1	7.70	-	-	17	5.89	-	-
2	5.90	-	-	12	5.38	-	-
1	5.22	-	-	100	5.13	-	-
1	5.12	-	-	33	5.01	-	-
18	4.91	-	-	56	4.82	-	-
2	4.60	-	-	22	4.48	-	-
3	4.08	-	-	43	4.39	-	-

Notes: No corresponding parameters between XRD and SAXS show with "-".

Table 2. SAXS and XRD comparison of liquid-crystal and crystal during avian development

## 4. Comparative study on mouse models of steatohepatitis and embryonic hepatic liquid crystal

### 4.1 High protein and fat diet mouse models

Using a diet high in protein and fat, a Steatohepatitis mouse model was generated. After three months, the liver of these animals turned light yellow and were larger in comparison to the liver of control animals. After 9 months, the livers were two to three times the size of mice being fed the normal diet. These livers also exhibited plaque and had significantly enlarged spleens. Using X-ray diffraction, small angle X-ray scattering, and phase transition, previous reports have characterized the distribution ultrastructure, and chemical composition of chicken hepatic LCLDs. Using this information and the well-established Steatohepatitis animal model, three conclusions were made: (1) The liquid-crystals in Steatohepatitis liver were produced in a pathological process similar to hepatic liquid-crystal formation in avian embryogenesis; (2) Small angle X-ray scattering revealed that liquid-crystal are distributed every 38Å in the hepatic tissue of Steatohepatitis animals. This distribution matched that reported in avian embryonic livers; (3) In Steatohepatitis animals, the liquid-crystals are distributed on the hepatic cords, which match the localization of liquid-crystals in embryonic liver.

## 4.2 Gene manipulation of related protein expression

Gene manipulation has become powerful tool for exploring gene function on molecular mechanisms of human diseases. In one instance, Shimano and colleges generated a transgenic mouse overexpressing truncated SREBP-1a (sterol-regulated proteolysis), a SREBP a membrane-bound transcription factor released by sterol-regulated proteolysis [Wang X *et al* 1994]. This gene abnormality resulted in lipid deposition in atherosclerotic lesions originating from liver accumulation of HLCDs [Shimano H *et al* 1996].

Stimulating a high level expression of the promoter in liver, the transgene was generated encoding the nuclear fragment of SREBP-1a, the most potent of the three SREBP isoforms. The truncated SREBP-1a is synthesized as a cytosolic protein instead of trafficking to cell surface, and it enters the nucleus without proteolysis, resulting in massive lipid overproduction. The livers of these transgenic mice dramatically enlarged and filled with fat, consisting of a cholesteryl esters and triglycerides mixture. The amounts of fat can be 5 to 25 fold higher than those observed in normal liver. The data on LDL receptors indicate that human steatohepatitis and atherosclerosis are linked diseases [Shimano H *et al* 1996]. Further studies have proven this biological mechanism to fit both alcoholic and non-alcoholic steatohepatitis within *in vitro* and *in vivo* studies [Horton JD *et al* 2002; Ji C *et al* 2006; You M *et al* 2002; Browning JD *et al* 2004].

## 4.3 Origins of fatty liver disease through hepatic liquid-crystals

In human, liver contains 4~6% fat, mostly made up of phospholipids, glycerides and cholesterol. When these fats accumulate in the liver, patients suffer from steatohepatitis or Hepatic steatosis (Fatty Liver Disease), which in turn causes liver enlargement, and abnormal liver function. Though Fatty Liver Disease is most common in overweight and diabetic patients [Hickman IJ *et al* 2007], a number of pathologic conditions such as excessive alcohol consumption or genetic disorder triggers this accumulation and will be followed by fibrosis and cirrhosis [Ban CR *et al* 2008; Preiss D *et al* 2008; Wilfred de Alwis NM *et al* 2008]. These associated illnesses make steatohepatitis a high mortality disease [El-Zayadi AR 2008; Xirouchakis E *et al* 2008]. Massive maltene-cross liquid-crystal droplets, like those found in embryonic liver, are observed in the biopsies of a large number of steatohepatitic patients. These hepatic liquid-crystal droplets (HLCDs) have been detected in large numbers of steatohepatitic patients through biopsy examination. Although their data has not yet been published, the phenomenon has been developed as a clinical examines procedure and filed for US documentation and invention disclosure.

## 4.4 Embryonic-like liquid-crystal linking steatohepatitis to atherosclerosis

Since the 1970s, liquid-crystalline structures have been observed in atherosclerotic lesions [Lang PD *et al* 1970, Saul S *et al* 1976]. The first investigation carried out on 95 individual atherosclerotic lesions obtained from 26 patients' classified the lesions into three groups, fatty streaks, fibrous plaques, and gruel (atheromatous) plaques. Using chromatography, the lipid composition of these lesions was determined to be cholesterol, cholesterol ester, and phospholipid. Using polarizing microscopy and X-ray diffraction, these lesion lipids were revealed to accumulate as liquid-crystals in lesions composed of special smooth foam cells [Saul S *et al* 1976, Kruth HS *et al* 2001]. This phenomenon was further confirmed by another

group [Goldstein JL *et al* 1977] and later mimicked *in vitro* [Joseph B *et al* 1984]. Analysis of familial hypercholesterolemia through *in vitro* fibroblast overloading experiments demonstrated that lipid deposition in the vascular wall is accomplished via low density lipoprotein-cholesteryl esters (LDL). These complexes are mediated by LDL receptors distributed on cell surfaces [Goldstein JL *et al* 2008; Brown MS *et al* 1974, 1975; Goldstein JL 1977 and 1979].

As discussed above, gain-of-function mutations containing overexpression of a truncated form of SREBP-1a links human steatohepatitis to atherosclerosis through LDL receptors. At this mutation results in liquid crystal depositions as part of the disease pathology, the finding directs a new prospect to exploring the biological function of liquid crystal. As a structure normally only found during embryogenesis, the existence of liquid crystals during disease biology suggests a new method approaching pathology from an embryological point of view. Further understanding of the role liquid crystals play in embryogenesis would doubtless reveal its role in pathogenesis of human diseases and help develop early diagnostics biophysics marker and more effective treatments.

## 5. Conclusion

Based on current discoveries obtained via XRD, SAXS, confocal microscope, and polarization microscopy in combination with cryo-section, push-release procedure for fluidity measurement, and thermal stage for phase transition progress has been made in the field of liquid crystal function in embryogenesis and pathogenesis of human diseases. With this methodology, the research has proved that, during the embryo development, liquid crystals are readily identifiable in the embryo through their Maltese-Cross birefringence texture. Liquid crystals with this configuration display strong fluidity accompanied with shape-changing properties under direct pressure conditions. XRD and SAXS analysis display a single-peak pattern corresponding to the Bragg distance of liquid crystal. Liquid crystals almost identical to those found in the developing embryo have been found in the affected tissue of multiple diseases.

Liquid crystal configuration within the embryo, animal disease models, and diseased human tissues are all cytoplasmic with Maltese-Crosses situated in cells of various tissues, especially in the luminal portion of kidney during diseases and embryonic blood. Further investigation into liquid crystal involvement in disease through its embryonic mechanisms is expected to generate new diagnostic protocols for liquid crystal related diseases, such as ARMD, Steatohepatitis, Atherosclerotic lesions, and Fabry-Anderson.

## 6. References

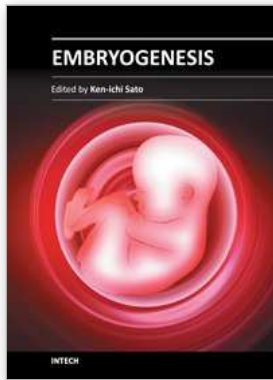
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