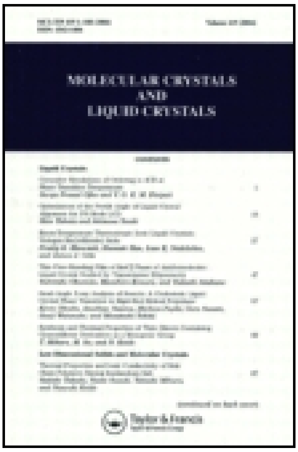

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Xue-Hong Xu [a] , Chun-Lin Wang [a] , Xi-Zhai Wu [a] & Hai-Ping He [a]

[a] School of Life Science, Wuhan University, Wuhan, 430072, Wuhan, P. R. China

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COMPARATIVE STUDIES ON THE HEPATIC LIQUID CRYSTAL LIPOID DROPLETS OF NEWBORN DUCKS AND NEWBORN PIGEONS

XUE-HONG XU CHUN-LIN WANG XI-ZHAI WU
HAI-PING HE
School of Life Science, Wuhan University, Wuhan 430072, Wuhan, P. R. China

Abstract We observed that the hapatic liquid crystal lipoid droplets (LCLD) of newborn ducks and newborn pigeons existed mainly in hepatic cells of hepatic cord and less in blood sinus, respectively with Bragg *d* values of 36. 8 Å and 38. 4 Å obtained by small-angle X-ray scattering (SAXS). X-ray difraaction (XRD) patterns made known that the fresh livers of newborn ducks and pigeons, dealt with freezing and thawing at room terperature, corresponded respectively to their own LCLD lipid crystals. The XRD data comparison indicated that the hepatic LCLD crystal of newborn pigeons was distinctively different from the hepatic LCLD crystal of newborn ducks and chick-embroyos. They belonged in two different kinds of crystal phases. But any one of them was phase-mixture and contained a little of the other, each other.

Key Words Liquid crystal lipoid droplet Liver Newborn duck Newborn pigeon

INTRODUCTION

Our former investigatins showed that dispersed liquid crystal droplets appered in many important organs and tissues at different stages during animal developement process and existed as the form of Malterse cross with strong birefringence between crossed polarisers. For instance, a large number of liquid crystal lipuid droplets (LCLD) appeared in the ootid of *Tilapia nilotica* ' s ootheca within oocyte becoming mature. [1] In the fat body in chrysalis of Chinese honey bee (*Apis cerana*), during the metamorphosis process, the protein granules largely had in reserve at liquid crystal-ordered state with a periodic spacing of about 44. 1 Å.[2] Particularly in chick developement process, the transitions from isotropic to anisotropic state took place in 18 kinds of important organs and tissues.[3,4] Especially, the LCLDs in the liver had a unique transition characteristic between both of LCLD, LCLD crystal and isotropic droplet.[5,6,7] These phenomena indicate that the appearance of liquid crystalline must play an important role in animal development.

In current work, the hepatic LCLDs in newborn ducks and pigeons were located in the hepatic cells of hepatic cord and less in blood sinus by polarised microscopy. The periodic spacings of the LCLDs were obained by directly detecting SAXS patterns of the fresh livers without any separation . The XRD data of the hepatic LCLD crystals and the crystals of extracted lipid from LCLDs of newborn duck and pigeon were compared .

MATERIALS AND METHODS

The Eggs of Beijing duck and domestic pigeon were incubated at routine conditions. Livers were drawn from the newborn individuals of 1-7 days old.

Polarized Microscopy

Fresh livers of newborn ducks and pigeons were cut on freezing microtone into 10-12 μm thickness sections. Frozen sections were mount on slide. A drop of glycerol-water solution (20%) was placed on the section and a cover glass was covered.

The distribution of LCLD crystals in liver tissue was observed under polarized microscope between crossed polarizers or non-crossed polarzers. The LCLD crystals counverted to isotropic droplets and then resumed LCLD while heating the section up to about 45℃ and cooling to room temperature.[5,8] The restated LCLDs in the section showed no difference from LCLDs in fresh liver smear under polorized microscope, so the distribution of LCLD crystals and the restated LCLDs in frozen section represented the distribution of LCLDs in liver tissue of newborn ducks and pigeons.

Small-Angle X-ray Scattering (SAXS)

SAXS patterns of fresh livers from newborn ducks and pigeons without any separation were taken on the small-angle goniometer of D/max-rA diffractometer in diffraction angle (2θ) of 0. 3-20 degree on the conditions of CuK_{α} radiation, Ni filter, powder of 50 kV x 100-120 mA and slits of 0. 16-0. 12-0. 2-0. 4 mm. Two pieces of non-diffraction membranes were fixed on both sides to refrain the samples from sliding and moisture-evaporating.

X-ray Diffraction (XRD)

XRD patterns of frozen and thawed fresh livers from newborn ducks and pigeons, in which LCLDs existed in cryctal state, were taken on the wide-angle goniometer of D/max-rA diffractometer in diffraction angle (2θ) of 1-20 degree on the conditions of CuK_{α} radiation, graphite monochromator and slits of 1/6°—1/6°—0. 15mm—0. 45mm.

The fresh livers were cut into small pieces and pulped after mixing equal volumes of the tissue and 0. 1 M phosphate buffer solution (pH 7. 4). The up-suspended substances of centrifugation of 22. 4 g for 10 — 15 min were LCLDs . Lipid components of LCLD extrated by Folch method. The XRD patterns of crystals of the extract were taken on as above.

The all XRD data were processed by the X-ray diffraction analysis software package written by the Materials Institute of Tsinghua University in diffraction angle (2θ) from 3 to 40 degree.

RESULTS AND DISCUSSION

Our past investigatins have proved that the LCLDs started to appear in the liver of 8 days old chick embryo, reached to a maxium amount at 17-18 days old and lasted for the first month on the postembryonic develoment at least.[3,5] Therefore , the samples in this paper got from 1-7 days old newborn ducks and pigeons.

Tissue-location of the hepatic LCLDs

Between crossed polarizers, rhombur-flake shaped and needle shaped crystals with strong birefringence exist in the liver-frozen sections of newborn duck and pigeon (Fig. 1a, 2a). Between non-crossed polarizers, the crystals are observed obvioulsy distributing in the hepatic cords (Fig. 1b, 2b). Heating the frozen section and then cooling to room temperature is accompanied by two types of transtions, the first being the crystal to disordered-isotropic lipoid droplets and the second being the isotropic lipoid droplets back to liquid crystal state lipoid droplets. The restated LCLDs are very similar to the LCLDs in liver smear under polaried microscope. (Fig. 1c, 2c)[9] Thus, it was determined that the hepatic LCLDs of newborn ducks and pigeons as the LCLD in chick embryo[5,7] distribute mainly in the hepatic cells of hepatic cord and less in blood sinus.

Comfirmation Of The Hepatic LCLD

SAXS patterns of the fresh livers of newborn ducks and pigeons show that there is only a small-angle reflection within the scattering-angle range (2θ) from 0.3°-20° (Fig. 3a, 4a). The small-angle reflection indicates that the Maltese cross droplets in the livers are in liquid crystalline state of an ordered-arrangment. The Bragg *d* values of the LCLDs in the livers of newborn duck and pigeon are 38.4 Å and 36.8 Å respectively. The LCLDs in two samples should be similar to the LCLDs in chick embryos[7] being smectic liquid crystal phase of concentric lamellas. There are no distinctively difference among the *d* values of the hepatic LCLDs in newborn ducks, newborn pigeons and chick embryos[5].

Diffraction Characteristics Of The LCLD Crystals

Figure 3b and 4b are the XRD patterns of the frozen and thawed livers form newborn ducks and pigeons. Figure 3c and 4c are the XRD patterns of the crystals of extracted lipid from newborn ducks' and pigeons' LCLDs. Pattern 3b to 3c, pattern 4b to 4c are correspondent elementarily, so we use pattern 3c and 4c indicate the diffraction characteristic of the LCLD crystals. Table 1 shows that the diffractions of newborn duck is evidently different from that of newborn pigeon but corresponds completely to the diffractions of chick embryo. A series of diffractions in the XRD pattern of newborn pigeon does not chime in with the diffractions of newborn duck and chick embryo. So, the LCLD crystal of newborn ducks and chick-embryos is a kind of crystal

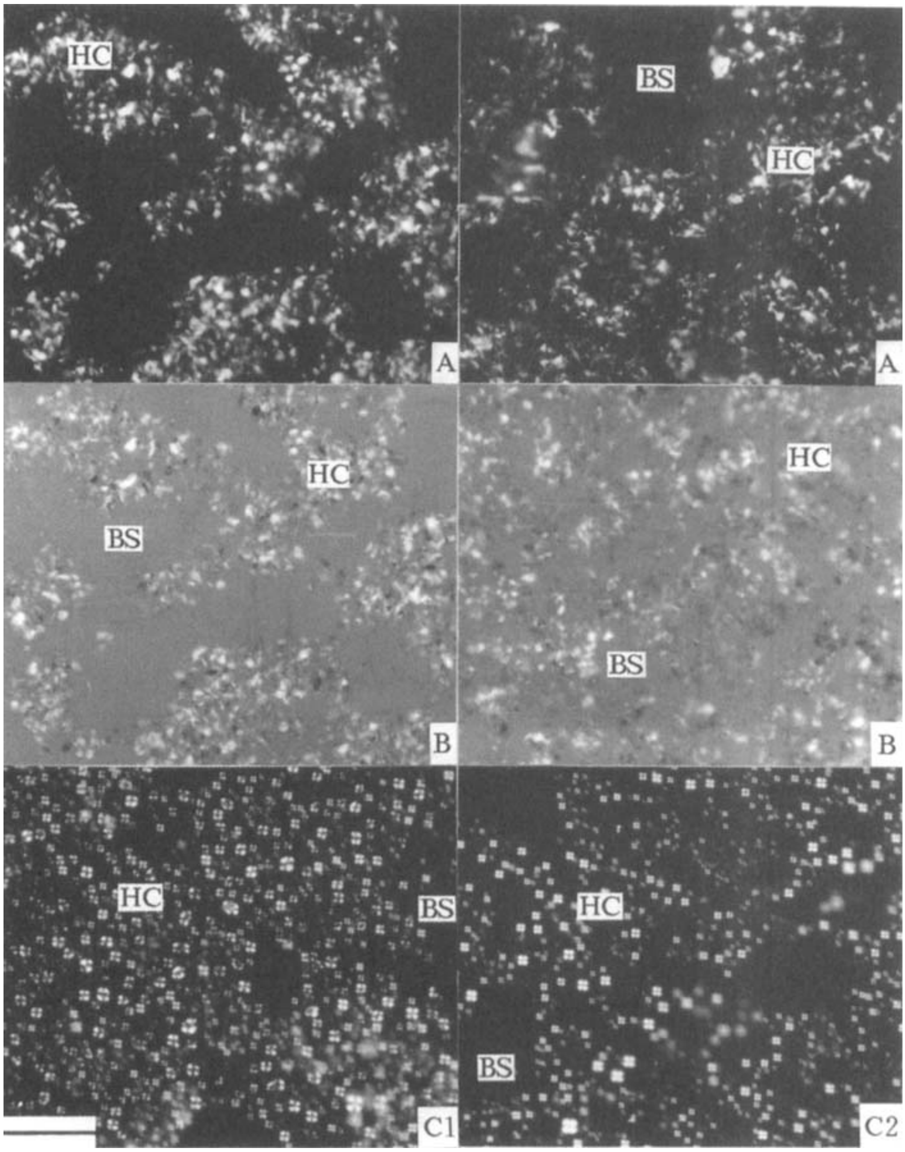


Fig. 1, 2 Microscopic viwes of thawed, frozen section of the fresh liver from 3 days old newborn duck (1a,b,c) and newborn pigeon (2a,b,c) at 18-20℃. A or B showes the LCLD crystals between crossed polarizers or non-crossed polarizers. C showes the restated LCLD. The position of blood sinus (BS) and hepatic cord (HC) are indicated. Scale bar is 90um.

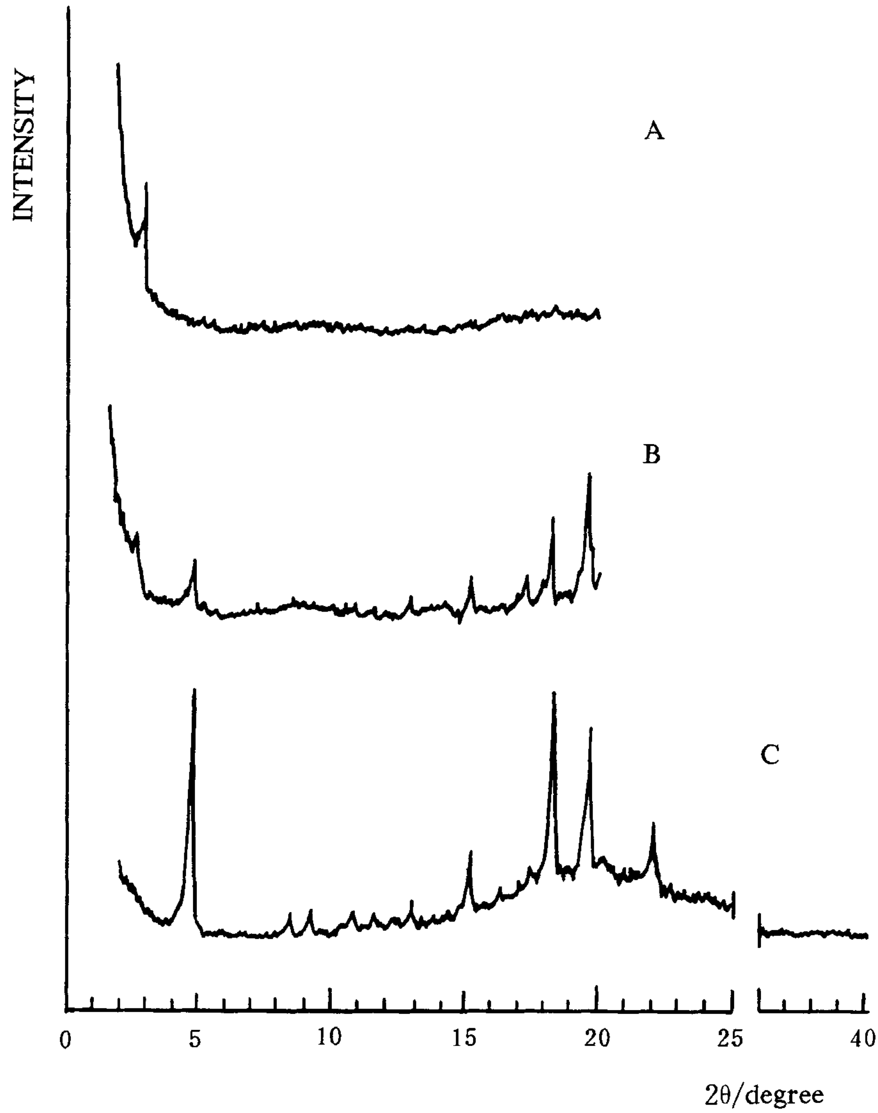


Fig. 3 Small angle X-ray scattering and X-ray diffraction patterns of different samples from a 7 days old newborn duck at 18-20℃. A, SAXS pattern of fresh liver with LCLD. B, XRD pattern of the frozen and thawed liver with crystals. C, XRD pattern of the crystal of extracted lipid from the LCLD.

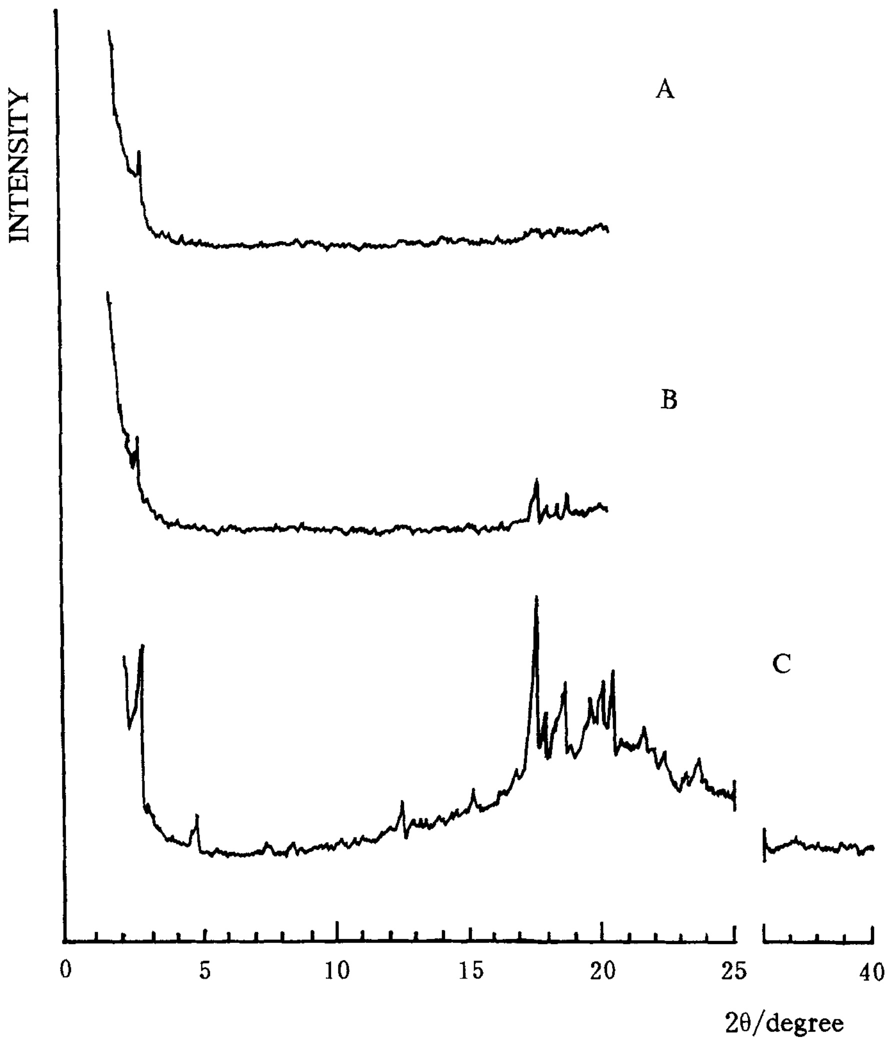


Fig. 4 Small angle X-ray scattering and X-ray diffraction patterns of different samples from a one days old newborn pigeon at 18-20℃. A, B and C are the same as the circumstances in figure 3 respectively.

TABLE 1 X-ray diffraction data of the frozen ,thawed liver tissues and the lipid crystal extracted from newborn ducks, pigeons and chick-embryos at 18—20℃

Newborn Chicken				Newborn Duck				Newborn Pigeon			
Thawed Fresh Liver Tissue		Lipid Crystal of LCLD		Thawed Fresh Liver Tissue		Lipid Crystal of LCLD		Thawed Fresh Liver Tissue		Lipid Crystal of LCLD	
I/I_0	d(Å)	I/I_0	d(Å)	I/I_0	d(Å)	I/I_0	d(Å)	I/I_0	d(Å)	I/I_0	d(Å)
100	19.32	100	19.49	100	19.32	100	19.32			32	19.48
										11	12.10
		3	17.11			8	17.05				
										10	10.71
		1	10.59			3	10.54				
		4	9.73	5	9.69	3	9.73				
						2	8.30				
		1	7.77			2	7.74				
										30	7.21
						1	6.96				
10	5.92	2	5.94	2	5.91	6	5.91			17	5.89
										12	5.38
		1	5.27			1	5.27				
15	5.18	1	5.13	2	5.17	2	5.12	100	5.13	100	5.13
						2	5.02	36	5.01	33	5.01
								52	4.83	56	4.82
36	4.90	11	4.91	7	4.90	28	4.90	24	4.89		
16	4.58	2	4.60			6	4.59				
										22	4.48
										43	4.39
15	4.06	3	4.09	4	4.08	8	4.08				

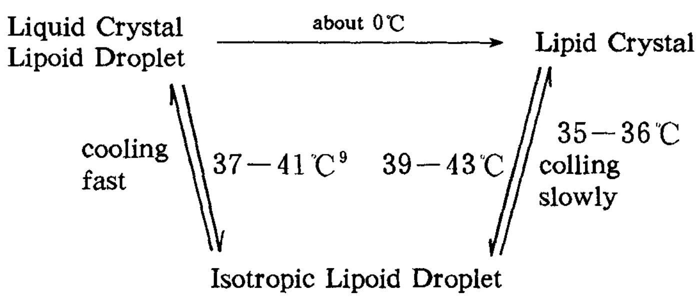
phase and the LCLD crystal of newborn pigeons is another kind of crystal phase. Then, both crystal phases are not a simple phase but two-phase mixture. Any one of two contains a little of the other because the strong diffractions of the first phase existing in the second and the strong diffractions of the second phase existing in the first.

We reported that during chick embryonic developement, liquid crystal state apppeared in 18 kinds of important organs and tissues such as yolk sac, liver, spleen, marrow, adrenal, thyroid gland, meso-and meta-nephros at different incubation day (Table 2).[3,10]

TABLE 2 Time-table of the liquid crystal appearance in organs and tissues while chick embryonic development[3,10]

Organ or Tissue	Incubation Day of Liquid Crystal Appearance	Organ or Tissue	Incubation Day of Liquid Crystal Appearance
Liver	8	Yolk Sac	2
Spleen	20	Production Gland	13
Bile	20	Mesonephros	17
Skin	10	Metanephros	17
Forclump of Brain	8	Marrow	20
Mesoplam	8	Heart (Blood)	19
Duodenum	17	Thyroid Gland	10
Nerve	14	Thymus Gland	20
Adrenal	13	Dark Area of Embryonic Disc	2.7

In 1991, we reported the unique characteristics of the hepatic LCLD transtions on the third China-Japan bilateral symposium on biophysics.[8] These are



Because the LCLD could exist in crystal state at room temperature, we found that there is a storage process of cholesteryl oleate in liquid crystal state during the chick embryo development by using XRD.[6] The massive cholesteryl oleate molleculars, which are dissolved in water, formed liquid crystal droplets with lecithin make themselves not to crystallize and destroy the embryonic hepatic cells. As a precursor of steroid hormones, the cholester l ester provide sufficient materials to synthesize male hormone, female hormone, calcium-mobilizine hormone and so on for tissue-construction of embryonic developemnt. The appearance of liquid crystalline state in so many organs and tissues within animal dovelopment is worthy to be studied further.

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