Synergism of Antithyroid Agent and Estrogen in the Induction of Experimental Fatty Livers in Growing Chicks

Yukio AKIBA, Kazuaki TAKAHASHI, Atsuro MATSUDA
and Tatsuro MATSUMOTO

Department of Animal Science, Faculty of Agriculture,
Tohoku University, Sendai 980, Japan

Excessive lipid deposition in liver is involved in fatty livers and fatty liver-hemorrhagic syndrome (FLHS) and is influenced by nutritional factors and metabolic states^{1,2)}. Recently, HAGHIGHI-RAD and POLIN³⁾ and AKIBA *et al.*⁴⁾ have indicated that enhanced lipid deposition in liver and hepatic hemorrhages are associated with high level of plasma estradiol in laying hens. This is confirmed by previous findings that estrogen administration induced hepatic steatosis in immature chicks⁵⁻¹¹⁾ and hepatic hemorrhages in immature chicks^{12,13)}.

Administration of antithyroid agent, such as propylthiouracil, induced lipid deposition in liver of immature chicks^{11,14-16)}. In the previous paper¹¹⁾ from this laboratory, an induction of excessive lipid deposition in liver was induced by synergetic action of antithyroid agent and estrogen in growing chicks.

The following experiment was conducted to study effects of graded doses of antithyroid agent and estrogen, and their interactions on lipid deposition in liver of growing chicks.

Materials and Methods

Ninty-six White Leghorn male chicks (14 days old) were divided into 16 groups of 6 chick each with 4×4 factorial arrangement. They were housed in wire cages under controlled light (14 hours light photoperiod) in air-conditioned room at 25°C and fed ad libitum a practical-type mash diet containing 20.7% CP and 2.9 kcal of metabolizable energy per gram for 13 days. Propylthiouracil (PTU, Sigma Chemical Company, USA) and dienestrol diacetate (DD, Sigma Chemical Company, USA) were added to the diet at levels of 0, 0.025, 0.05 and 0.10% and at levels of 0, 0.04, 0.08 and 0.16%, respectively.

Eight hours after deprivation of the diet, blood was collected by cardiac puncture with a heparinized syringe prior to slaughter and centrifuged for 15 minutes at 1,500×g and the plasma was stored at -20°C for later analysis. Liver lipid was extracted by the method of FOLCH *et al.*¹⁷⁾ and determined gravimetrically. Total lipid content in plasma was determined by the method of BRAGDON¹⁸⁾ and triglyceride content in the liver and plasma was determined by the method of FLETCHER¹⁹⁾. Activity of glutamate oxalacetate transaminase (GOT) in plasma was determined by a modified method of REITMAN and FRANKEL²⁰⁾, using colorimetric measuring kits (Wako Pure Chemical Industries, Japan). The data were subjected to analysis of variance²¹⁾.

Results

Table 1 shows feed intake, body weight gain and liver weight. Feed intake was increased by administration of DD. The increased feed intake by DD appears to be reduced with increasing PTU in the diet. Body weight gain during 13 days was increased by DD and lowered by PTU. The maximal body weight gain was demonstrated in chicks given 0.025% PTU and 0.04% DD, concomitantly, and it was lowered with increasing DD and PTU in the diet as shown in Fig. 1A. Liver weight was increased with increasing DD in the diet and concomitant addition of PTU (0.025%) induced further enlargement of liver, though administration of PTU over 0.05% was less effective (Fig. 1B).

Total lipid and triglyceride contents in the liver are presented in Table 2 and Figs. 1C and 1D. PTU without a concomitant administration of DD had no influence on hepatic lipid content (wet %). Concurrent administration of PTU and DD over the level of 0.025% and 0.04%, respectively, resulted in a significant increase of the liver lipid content. An increasing effect in liver lipid deposition (mg/liver) by DD was obviously demonstrated and it was extensively augmented by a concomitant administration of PTU at 0.025%. Effect of

Table 1. Effects of administration of PTU and/or DD on feed intake, body weight gain and liver weight in chicks¹

Treatment ²		Feed intake (g)	Body weight gain (g)	Liver weight	
PTU (% in diet)	DD			(g)	(g/100 g BW)
0	0	420	168±11 ^{def3}	8.0±1.0 ^h	2.68±0.21 ⁱ
0	0.04	499	173±3 ^{def}	8.4±1.4 ^h	2.75±0.43 ⁱ
0	0.08	474	193±12 ^c	11.6±1.8 ^g	3.57±0.61 ^h
0	0.16	480	199±23 ^b	18.1±2.4 ^d	5.45±0.72 ^e
0.025	0	487	163±29 ^{f g}	10.7±1.4 ^g	3.67±0.61 ^h
0.025	0.04	479	211±16 ^a	18.1±4.4 ^{cd}	5.27±1.08 ^{ef}
0.025	0.08	487	206±20 ^{ab}	22.7±1.6 ^b	6.71±0.36 ^c
0.025	0.16	444	176±16 ^d	24.2±4.2 ^a	8.11±1.28 ^a
0.05	0	372	143±8 ^{gh}	10.2±0.8 ^g	3.61±0.31 ^h
0.05	0.04	420	166±24 ^{ef g}	13.6±2.8 ^f	4.50±0.65 ^g
0.05	0.08	484	178±12 ^d	19.3±2.1 ^c	6.33±0.45 ^d
0.05	0.16	408	154±20 ^g	20.5±4.2 ^c	7.25±1.01 ^b
0.10	0	418	140±14 ^h	10.3±0.6 ^g	3.84±0.20 ^h
0.10	0.04	424	172±8 ^{def}	14.8±1.3 ^g	4.87±0.48 ^{f g}
0.10	0.08	436	159±25 ^{f g}	16.2±2.5 ^e	5.55±0.41 ^e
0.10	0.16	413	156±23 ^{f g}	18.7±2.5 ^d	6.59±0.81 ^{cd}
Analysis of variance					
PTU			P<0.01	P<0.01	P<0.01
DD			P<0.01	P<0.01	P<0.01
PTU×DD			P<0.01	P<0.01	P<0.01

¹ Chicks were fed diet added PTU and/or DD for 13 days from 14th day of age.

² Abbreviations: PTU, propylthiouracil; DD, dienestrol diacetate.

³ Mean±SD of 6 observations. Means with different superscripts in columns are significantly (P<0.05) different.

Table 2. Effects of administration of PTU and/or DD on total lipid and triglyceride contents in liver of chicks

Treatment		Liver total lipids		Liver triglyceride	
PTU (% in diet)	DD	(% wet weight)	(mg/liver)	(% total lipids)	(mg/liver)
0	0	4.5±0.3 ¹	369± 53 ^h	27.4± 9.0 ^e	99± 36 ^f
0	0.04	4.6±0.3 ⁱ	379± 38 ^h	37.1± 2.3 ^{cde}	141± 18 ^{ef}
0	0.08	6.2±1.3 ^e	735± 243 ^{fgh}	47.7±10.0 ^{bc}	349±146 ^{de}
0	0.16	13.9±1.5 ^a	2510± 394 ^b	64.4± 5.4 ^a	1626±333 ^a
0.025	0	4.7±0.4 ^{hi}	503± 87 ^{gh}	34.2±10.9 ^{cde}	174± 63 ^{ef}
0.025	0.04	8.9±3.1 ^c	1457± 549 ^{cde}	60.0±17.7 ^{ab}	1078±756 ^{bc}
0.025	0.08	12.5±3.2 ^b	2825± 788 ^{ab}	72.1± 5.7 ^a	1942±519 ^a
0.025	0.16	13.3±3.6 ^{ab}	3258±1490 ^a	65.1±12.6 ^a	2073±996 ^a
0.05	0	4.8±0.7 ^{chi}	495± 109 ^{gh}	29.7±11.7 ^{de}	156± 96 ^{ef}
0.05	0.04	6.1±1.4 ^{eg}	852± 342 ^{efgh}	43.8±18.7 ^{bcd}	417±266 ^{de}
0.05	0.08	8.6±1.5 ^c	1685± 412 ^{cd}	64.5± 7.1 ^a	1079±248 ^{bc}
0.05	0.16	8.4±2.4 ^{cd}	1796± 942 ^c	59.8±15.0 ^{ab}	1157±871 ^b
0.10	0	4.5±0.2 ⁱ	466± 37 ^{gh}	24.0± 7.3 ^e	113± 39 ^f
0.10	0.04	7.2±1.9 ^{de}	1077± 310 ^{cdefg}	36.8±13.1 ^{cde}	418±215 ^{de}
0.10	0.08	7.6±2.7 ^{cd}	1276± 641 ^{cdef}	45.9±18.3 ^{bcd}	680±599 ^{cd}
0.10	0.16	6.6±1.0 ^{de}	1320± 293 ^{cdef}	45.9± 6.5 ^{bcd}	613±191 ^{cd}
Analysis of variance					
PTU		P <0.01	P <0.01	P <0.01	P <0.01
DD		P <0.01	P <0.01	P <0.01	P <0.01
PTU × DD		P <0.01	P <0.01	P <0.01	NS ²

¹ Mean±SD of 6 observations. Means with different superscripts in columns are significantly (P<0.05) different.

² Not significant.

DD was proportionally reduced with increasing PTU over 0.025%. The maximal deposition of lipids in the liver was obtained by concurrent administration of PTU (0.025%) and DD (0.08~0.16%). Similarly hepatic triglyceride content was increased by the administration of DD (0.16%) or concurrent administration of PTU and DD but it was not increased by PTU, per se.

Total lipid and triglyceride contents and GOT activity in plasma are shown in Table 3 and Figs. 1E and 1F. Total lipid (Fig. 1E) and triglyceride in the plasma are increased by PTU and were extensively increased by DD feeding. Marked increases of lipid and triglyceride contents in the plasma were demonstrated in the chicks administered PTU and DD, concurrently. And those were maintained even when level of PTU in the diet increasing, but this trends are clearly different from that in the liver lipid and triglyceride contents. Plasma GOT activity was elevated by administration of PTU but it was not affected by DD. Concomitant administration of DD in addition to PTU increased plasma GOT activity (Fig. 1F).

Fig. 1. Body weight gain, liver weight, total lipid and triglyceride contents in the liver, plasma lipids and plasma GOT activity influenced by administration of PTU and/or DD in chicks.

Table 3. Effects of administration of PTU and/or DD on total lipid and triglyceride contents and GOT activity in plasma of chicks

Treatment		Plasma		
PTU (% in diet)	DD	Total lipid (mg/100 ml)	Triglyceride (mg/100 ml)	GOT ¹ (Karmen unit)
0	0	327 ± 84 ^{d2}	69 ± 9 ^f	72 ± 5 ^{ef}
0	0.04	364 ± 175 ^{cd}	161 ± 49 ^e	71 ± 5 ^{ef}
0	0.08	1313 ± 875 ^{bc}	678 ± 283 ^d	66 ± 5 ^f
0	0.16	3868 ± 2439 ^b	2450 ± 931 ^{cd}	72 ± 6 ^{ef}
0.025	0	534 ± 355 ^c	215 ± 64 ^e	83 ± 19 ^{def}
0.025	0.04	3612 ± 2616 ^b	1827 ± 1353 ^{cd}	96 ± 18 ^{cde}
0.025	0.08	9992 ± 2939 ^a	6048 ± 1978 ^b	125 ± 47 ^{bc}
0.025	0.16	11063 ± 4110 ^a	9426 ± 1405 ^a	169 ± 40 ^a
0.05	0	574 ± 141 ^c	214 ± 25 ^e	106 ± 19 ^{cd}
0.05	0.04	3047 ± 2457 ^b	1670 ± 1248 ^d	108 ± 32 ^{cd}
0.05	0.08	9157 ± 2488 ^a	6769 ± 2424 ^b	121 ± 29 ^{bc}
0.05	0.16	12607 ± 3929 ^a	9065 ± 3041 ^a	143 ± 31 ^b
0.10	0	767 ± 233 ^c	341 ± 149 ^e	106 ± 26 ^{cd}
0.10	0.04	4202 ± 2594 ^b	3097 ± 1929 ^c	108 ± 21 ^{cd}
0.10	0.08	8685 ± 1611 ^a	6765 ± 2123 ^b	123 ± 21 ^{bc}
0.10	0.16	11538 ± 1145 ^a	9272 ± 855 ^a	125 ± 26 ^{bc}
Analysis of variance				
PTU		P < 0.01	P < 0.01	P < 0.01
DD		P < 0.01	P < 0.01	P < 0.01
PTU × DD		P < 0.01	P < 0.01	P < 0.01

¹ Abbreviation: GOT, glutamate-oxalacetate transaminase activity.

² Mean ± SD of 6 observations. Means with different superscripts in columns are significantly (P < 0.05) different.

Discussion

The injection of estrogen has been confirmed to induce hepatic steatosis and hyperlipidemia in immature chicks⁵⁻¹¹⁾. However, effect of oral administration of estrogen on the hepatic lipid content is not conclusive since LORENZ²²⁾ reported a small increase in liver lipid caused by dietary DD, whereas WOLFORD²³⁾ and ROBERSON and TRUJILLO²⁴⁾ stated that liver lipid was not elevated by feeding DD at 0.007% in the diet of laying hens. The present study, however, clearly proved that feeding DD over 0.08% in the diet caused a pronounced hepatic steatosis which is associated with hyperlipidemia and increase in feed intake and body weight gain in accordance with our previous paper¹¹⁾. Additionally, it is noteworthy that the extent of increase in the plasma lipid by feeding DD is more pronounced than that in the liver lipid (Table 2 and 3), indicating that DD stimulates not only overproduction of lipid in the liver but also secretion of the lipid from the liver in chicks.

It has been shown in immature chicks that administration of PTU induced hypothyroidism^{25,26)} and thereby resulting in enlargement of thyroid gland and an excessive lipid deposition in liver^{11,14-16)}. The increase of liver lipid by PTU feeding was also confirmed in the present study but it was less striking compared with the other findings¹⁴⁻¹⁶⁾ and was not fortified with increasing PTU in the diet. This could be ascribed to less feed intake in chicks fed higher levels of PTU (Table 1) because energy intake has shown to be involved in regulation of hepatic lipid deposition^{27,28)}.

Plasma transaminase activity has been ascertained to be elevated when liver impairment is artificially induced by administration of carbon tetrachloride^{29,30)} and force-feeding a high energy diet³¹⁾ in chicks. Plasma GOT activity, in this experiment, was increased by feeding PTU and further intensified by concomitant feeding with DD, suggesting that treatments with PTU or PTU+DD induced liver dysfunction accompanied with the hepatic steatosis in growing chicks.

Marked lipid deposition in the liver and hyperlipidemia were caused by the concomitant feeding of DD with 0.025% PTU and were mainly due to overproduction of triglyceride (Fig. 1). This evidence confirms our previous findings¹¹⁾ that hepatic steatosis and hyperlipidemia were caused by synergetic action of PTU and DD. Some of chicks given both 0.025% PTU and DD (0.08~0.16%) had hepatic hemorrhages, indicating that experimental fatty liver with hemorrhages could be induced by concurrent administration of PTU and DD in growing chicks, and also suggesting that this experimental fatty liver would be useful as a laboratory model for the detection of fatty liver-preventing factors.

The excessive lipid deposition in the liver induced by concurrent administration of PTU (0.025%) and DD was proportionally reduced with increasing PTU in the diet, though plasma lipid and triglyceride contents were maintained even when increasing PTU in diet. It was already suggested in our previous paper¹¹⁾ that feeding of PTU at a low level (0.025%) in the diet may retard secretion of lipid from the liver. But in the present experiment, it seems likely that high levels of PTU (over 0.025%) in the diet rather enhance secretion of lipids which was excessively produced in the liver by feeding DD, and thereby resulting in the gradual decrease of liver lipid. The reason for these phenomena are not well understood, but these observations could be supported by the findings in rats³²⁾

that output of triglyceride from perfused liver was accelerated by the injection of PTU.

Summary

Lipid and triglyceride content in liver and plasma and plasma transaminase activity were determined in 14-day-old male chicks given graded levels of propylthiouracil (PTU, 0, 0.025, 0.05 and 0.10%) and/or dienestrol diacetate (DD, 0, 0.04, 0.08 and 0.16%) in diet with 4×4 factorial arrangement for 13 days. Plasma transaminase activity was increased by feeding PTU or PTU+DD. Feeding PTU or DD induced significant increase of liver weight and lipid and triglyceride contents in the liver and plasma. Marked hepatic steatosis and hyperlipidemia were caused by concurrent administration of PTU (0.025%) and DD (0.08~0.16%) and those were gradually reduced with increasing PTU in the diet. It is suggested that experimental fatty livers are induced by synergetic actions of antithyroid agent and estrogen in growing chicks.

Literature

- 1) BUTLER, E.J.: *Avian Pathol.*, **5**, 1, 1976.
- 2) JENSEN, L.S.: *Fed. Proc.*, **38**, 2631, 1979.
- 3) HAGHIGHI-RAD, F. and D. POLIN: *Poult. Sci.*, **60**, 2278, 1981.
- 4) AKIBA, Y., L. S. JENSEN, C. R. BARB and R.R. KRAELING: *J. Nutr.*, **112**, 299, 1982.
- 5) LORENZ, F.W.: *Vit. Horm.*, **12**, 235, 1954.
- 6) BALNAVE, D.: *J. Endocrinol.*, **42**, 119, 1968.
- 7) KUDZMAN, D.J., P.M. HAGSTAD and R.E. STOLL: *J. Clin. Invest.*, **22**, 423, 1973.
- 8) YU, Y-L. and R.R. MARQUARDT: *Comp. Biochem. Physiol.*, **46 B**, 749, 1973.
- 9) BALNAVE, D. and J. PEARCE: *J. Endocrinol.*, **61**, 29, 1974.
- 10) PEARCE, J. and D. BALNAVE: *Biochem. Pharmacol.*, **24**, 1843, 1975.
- 11) AKIBA, Y., K. TAKAHASHI, M. KIMURA, S. HIRAMA T. MATSUMOTO: *Br. Poult. Sci.* (in press).
- 12) PEARSON, A.W. and E.J. BUTLER: *Res. Vet. Sci.*, **24**, 82, 1978.
- 13) POLIN, D. and J.H. WOLFORD: *J. Nutr.*, **107**, 873, 1977.
- 14) EVANS, J.D., N.O. BURYK, P.J. MIDDLETON and H.G. SCHWARTS: *Endocrinol.*, **70**, 465, 1962.
- 15) RAHEJA, K.L., J.D. SNEDECOR and R.A. FREEDLAND: *Com. Biochem. Physiol.*, **39 B**, 833, 1971.
- 16) RAHEJA, K. L. and W.G. LINSCHER: *Comp. Biochem. Physiol.*, **61 A**, 31, 1978.
- 17) FOLCH, J., M. LEES and G.H. SLOAN-STANLEY: *J. Biol. Chem.*, **226**, 497, 1957.
- 18) BRAGDON, J.H.: *Lipids and the Steroid Hormones in Clinical Medicine*. SUNDERMAN, F.W. and F.W. SUNDERMAN eds., pp 9, Lippincott, Philadelphia and Montreal, 1960.
- 19) FLETCHER, M.J.: *Clin. Chim. Acta*, **22**, 393, 1968.
- 20) REITMAN, S. and S. FRANKEL: *Am. J. Clin. Pathol.*, **28**, 56, 1957.
- 21) SNEDECOR, G.W. and W.G. COCHRAN: *Statistical Methods*, 6th ed., The Iowa University Press, USA, 1967.
- 22) LORENZ, F.W. and G.H. BACHMAN: *Poult. Sci.*, **26**, 419, 1947.
- 23) WOLFORD, J.H.: *Poult. Sci.*, **50**, 1331, 1971.
- 24) ROBERSON, R.H. and V. TRUJILLO: *Poult. Sci.*, **54**, 715, 1975.
- 25) AKIBA, Y. and T. MATSUMOTO: *Jpn. J. Zootech. Sci.*, **48**, 757, 1977.
- 26) AKIBA, Y. and T. MATSUMOTO: *Jpn. J. Zootech. Sci.*, **49**, 61, 1978.
- 27) AKIBA, Y., A. HORIGANE and T. MATSUMOTO: *Jpn. J. Zootech. Sci.*, **49**, 54, 1978.
- 28) WOLFORD, J.H. and D. POLIN: *Poult. Sci.*, **53**, 65, 1974.
- 29) FOWLER, J.S.L.: *J. Com. Pathol.*, **80**, 465, 1970.
- 30) NARASHIMHAN, T.R. and S.G. NAIR: *Acta Vet.*, **19**, 269, 1974.
- 31) AKIBA, Y. and T. MATSUMOTO: *J. Nutr.*, **110**, 1112, 1980.
- 32) KEYS, W.G. and M. HEIMBERG: *J. Clin. Invest.*, **64**, 182, 1979.

鶏ヒナの実験的脂肪肝形成に対する抗甲状腺物質 とエストロジェンの相乗作用

秋葉 征夫・高橋 和昭・松田 敦郎・松本 達郎

東北大学農学部, 仙台市 980

鶏の実験的脂肪肝の形成に対する抗甲状腺物質とエストロジェンの作用と、それらの相乗作用について検討した。14日齢の白色レグホーン種雄ヒナを供試して、抗甲状腺物質 propylthiouracil (PTU) および合成エストロジェン dienestrol diacetate (DD) を飼料に混合して13日間給与した。試験区として、PTU 投与4レベル (0, 0.025, 0.05, 0.10%) および DD 投与4レベル (0, 0.04, 0.08, 0.16%) を組合わせた16区を設定し、各区に6羽を割り当てた。

増体量は PTU 投与で減少し、DD 投与で増加した。DD による増体量の増加は PTU 0.025% の同時投与でさらに亢進した。肝臓重量は PTU 投与で若干増加した。DD 投与ではその投与量に比例して肝臓重量が増加する傾向がみられ、それは PTU の同時投与により著し

く亢進した。肝臓中脂質およびトリグリセリド量は、DD の投与量に比例して増加し、さらに PTU 0.025% の同時投与で著しく増加した。しかし、PTU の投与量をさらに増すと、それらは減少する傾向にあった。PTU 0.025% および DD 0.08~0.16% の相乗効果により、肝臓脂質量は対照区の約10倍に増加し、一部の肝臓には出血点も認められた。血漿脂質およびトリグリセリド量は PTU+DD 投与により著しく増加し、高脂血症を呈した。血漿中トランスアミナーゼ活性は、PTU 0.025% と DD 0.08~0.16% の同時投与により著しく上昇し、肝機能の低下が推察された。以上のことから、鶏ヒナを用いて、抗甲状腺物質とエストロジェンの相乗作用による脂肪肝をつくり出すことが可能である。

(家禽会誌, 19, 238~244, 1982)