In 1966, a new acute form of myocardial disease with an alarmingly high mortality rate (40-50%) has been reported among chronic beer drinkers in Quebec City, Canada and in Omaha, Nebr. U.S.A. The etiology of the disease was suggested following the examination of beer, in which 1.2 ppm of cobalt was added in order to stabilize the foam and by the autopsy of victims, whose hearts contained ten times more cobalt than normal.

It has been shown that cobalt has two major effects on the rabbit. One effect was heart failure, which was similar to the finding in the beer drinkers of Omaha and Quebec. Another major effect was lipid-mobilizing action. The administration of cobalt chloride to rabbits resulted in a great increase in total serum lipids, especially most consistently and to the greatest extent in the increase of triglyceride. Previous workers have postulated that this hypertriglyceridemia results from cobalt damage to the alpha cells of the pancreas, or from sustained release into the circulation of increased amounts of lipid mobilizer, or from cobalt inhibition to the oxidation of pyruvate or fatty acid. McDermott et al reported that myocardopathy, marked elevation of the serum glutamic oxaloacetic acid transaminase (GOT) and lactic dehydrogenase (LDH) levels, as well as severe lactic acid acidosis were seen in the patients admitted to the Omaha Hospital.

The present investigation was done to determine whether the cobalt acts to raise the free fatty acid (FFA) level in blood plasma through the elevation of hormone sensitive lipase activity in adipose tissue and to accumulate the triglyceride in the liver, or inhibits the post heparin plasma lipoprotein lipase activity and consequently brings about the hypertriglyceridemia. Serum LDH activity and its isozyme were measured in relation with the action of cobalt to induce the accumulation of lactate or pyruvate through the inhibition of keto-acid oxidation.

**MATERIALS AND METHODS**

Male white rabbits varying in weight between 3 and 4 kg were used for this study. These animals were fed and allowed water ad libitum. Eleven rabbits were treated with a daily intramuscular injection of 10% solution of cobalt chloride in a dose of 25 mg/kg of body weight for seven consecutive days. Blood was collected from the ear vein before the primary injection of cobalt and by cardiac puncture on the 8th day of the experiment. Eleven animals were kept as control and bled before and after a 48 hr. starvation period.

After the first sample of blood was collected, 0.5 ml of 500 I.U. heparin solution was injected rapidly into the ear vein. A second sample of blood was drawn approximately three minutes after the heparin injection, and the specimens were prepared for the post-heparin plasma.

The animals were sacrificed by ear vein injection of air. Liver, kidney and perirenal adipose tissue were removed and stored at -20°C until use. Five hundred mg of perirenal adipose tissue, which was quickly removed after sacrifice, was homogenized by hand in a glass tissue
grinder with 4 ml of 0.154 M KCl. The assays of hormone sensitive lipase were performed in 12 ml glass-stoppered centrifuge tubes; 0.2 ml of homogenate was added to 0.8 ml of substrate, which contained in each 1 ml 30 mg of bovine serum albumin and 20 μ moles of sodium phosphate buffer, pH 7.0. The tube was incubated at 37°C and 0.1 ml of incubation mixture was offered for the determination of liberated free fatty acid every five minutes. Lipase activity is indicated as microequivalents of free fatty acid produced per g of tissue (wet weight) per hour of incubation.

Five hundred mg of perirenal adipose tissue was homogenized in chloroform-methanol solution and the filtrate was offered for the determination of FFA.

Five hundred mg each of liver and kidney was homogenized in chloroform-methanol and the filtrate was washed with 0.58% NaCl solution. The lower phase of the mixture was subjected to the determination of triglycerides as described by Folch et al. 

Thirty six adult male rats of Wistar strain, weighing more than 200 g were used in the study. They were fed on rat laboratory chow and allowed water ad libitum. Eighteen rats received an aqueous solution of cobalt sulfate (4 mg Co++/kg body weight) by intraperitoneal injection daily for 8 days. Eighteen treated and eighteen control animals were bled by heart puncture and the blood was analyzed for pyruvate and lactate concentrations.

Triglycerides in plasma and tissues were estimated by the modified method of Van Handel and Zilversmidt. Plasma and adipose tissue FFA were determined by the method of Laurell and Tibbling. Blood lactate was determined by the enzymatic method and blood pyruvate by the method of Friedmann and Haugen.

The total serum lactic dehydrogenase activity was measured by the spectrophotometric method described by Wroblewski and LaDue. Serum LDH was separated into five isoenzyme components by agar-gel electrophoresis as described by Wieme with only minor modification and stained with nitro blue tetrazolium dye. Quantitative determination of stained gel electrophoresis was done by densitometry of the isozyme bands.

**RESULTS**

Table 1 shows the comparison of lipid levels and the enzymatic activities in plasma and tissue between the cobalt treated and control rabbits. It can be seen from this table that seven successive days' injection caused about a seventeen fold increase in plasma triglyceride. However, the level of plasma FFA, the content of FFA and the activity of hormone sensitive lipase in perirenal adipose tissue, as well as the content of triglyceride in liver or kidney in the treated rabbits did not differ from the control. The post-heparin lipoprotein lipase activity in plasma of rabbits treated with cobalt was lower than the control, but for the sake of a small number of experimental animals, the difference was not statistically significant.

As shown in table 2, lactate concentration in plasma of the treated rabbits was significantly higher than that of the control, but the pyruvate concentration in the treated animals was not different from the control.

Table 3 shows that the intraperitoneal injection of cobalt sulfate into rats produced a significantly higher pyruvate concentration. On the contrary, the plasma lactate concentration in the treated animals was not different from the control.

More than a three-fold activity was noted in the serum LDH of rabbits treated with cobalt chloride compared with that of the control. An extreme rise in LDH-5 and a fall of LDH-1 was evidenced in the pattern of LDH isozyme in the treated rabbits. When total activity of LDH is multiplied by each isozyme percent, each isozyme activity in the treated group becomes far higher than that of the control group and an especially high activity is noticed in LDH-5.
These studies demonstrate a remarkable rise in plasma triglyceride concentration 7 days after a daily successive injection of cobalt into rabbits. The mechanism responsible for the development of hypertriglyceridemia after the injection of cobalt has not been established. It is unlikely that hypertriglyceridemia is due solely to increased mobilization of FFA from adipose tissue to the liver,¹⁹⁻²¹ since the level of plasma FFA was not elevated. The low level of plasma FFA corresponds with a minor content of FFA as well as low activity of hormone sensitive lipase in the perirenal adipose tissue. These facts provide evidence, which tends to deny the assumption that cobalt influences the adipose tissue function by stimulating the lipolytic activity of the enzyme.

** Table 1. Plasma lipid concentration, tissue lipid content and enzymatic activity of adipose tissue and plasma in control and cobalt chloride treated rabbits.**

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>FFA</th>
<th>Hormone Sensitive Lipase</th>
<th>Post-heparine LPL in Blood Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>plasma</td>
<td>liver</td>
<td>kidney</td>
<td>plasma</td>
</tr>
<tr>
<td></td>
<td>mg/dL</td>
<td>mg/g-tissue</td>
<td>mg/g-tissue</td>
<td>μEq/L</td>
</tr>
<tr>
<td>CoCl₂</td>
<td>(11)**</td>
<td>455.9±255.0</td>
<td>4.17±2.60</td>
<td>1.03±0.41</td>
</tr>
<tr>
<td>Control</td>
<td>(11)</td>
<td>25.9±18.6</td>
<td>6.52±2.88</td>
<td>1.00±0.84</td>
</tr>
</tbody>
</table>

** Significant at the 1% level. Values are mean±standard deviation. Figures shown in parentheses are number of rabbits.

** Table 2. The effect of cobalt on the blood lactate and serum pyruvate concentration in rabbits.**

<table>
<thead>
<tr>
<th></th>
<th>Lactate mg/dL</th>
<th>Pyruvate mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoCl₂</td>
<td>(8)** 32.7±7.6</td>
<td>(9) 4.31±1.90</td>
</tr>
<tr>
<td>Control</td>
<td>(9) 16.9±6.6</td>
<td>(11) 4.97±2.50</td>
</tr>
</tbody>
</table>

** Significant at the 1% level. Values are mean±standard deviation. Figures shown in parentheses are number of rabbits.

** Table 3. The effect of cobalt on the blood lactate and serum pyruvate concentration in rats.**

<table>
<thead>
<tr>
<th></th>
<th>Lactate mg/dL</th>
<th>Pyruvate mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoSO₄</td>
<td>(18) 12.96±7.17</td>
<td>(18)* 2.90±0.82</td>
</tr>
<tr>
<td>Control</td>
<td>(18) 9.87±7.37</td>
<td>(16) 2.26±0.87</td>
</tr>
</tbody>
</table>

* Significant at the 5% level. Values are mean±standard deviation. Figures shown in parentheses are number of rats.

** Table 4. The effect of cobalt on the LDH activity and the pattern of isozyme in rabbit serum.**

<table>
<thead>
<tr>
<th></th>
<th>Total Activity (units)</th>
<th>LDH Isozyme (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LDH-1</td>
</tr>
<tr>
<td>Treated</td>
<td>(10)** 287±65</td>
<td>26.9</td>
</tr>
<tr>
<td>Control</td>
<td>(10) 79±23</td>
<td>49.9</td>
</tr>
</tbody>
</table>

** Significant at the 1% level. Figures in parentheses are number of rabbits. Values are mean±standard deviation.

** DISCUSSION **

These studies demonstrate a remarkable rise in plasma triglyceride concentration 7 days after a daily successive injection of cobalt into rabbits. The mechanism responsible for the development of hypertriglyceridemia after the injection of cobalt has not been established. It is unlikely that hypertriglyceridemia is due solely to increased mobilization of FFA from adipose tissue to the liver,¹⁹⁻²¹ since the level of plasma FFA was not elevated. The low level of plasma FFA corresponds with a minor content of FFA as well as low activity of hormone sensitive lipase in the perirenal adipose tissue. These facts provide evidence, which tends to deny the assumption that cobalt influences the adipose tissue function by stimulating the lipolytic activity of the enzyme.
After seven consecutive days' injection, rabbits fell into a state of anorexia, ate nothing and were similar in appearance due to deprivation of food. It has been shown that in the fasting state, when no exogenous fat is injected, almost all circulating triglycerides are of hepatic origin. As stated above the increased mobilization of FFA from adipose tissue to the liver was not evidenced at the time of sacrifice, so that hypertriglyceridemia could be provoked by other factors as follows: i) increase in the rate and amounts of triglyceride synthesis in the liver, ii) release of triglycerides from the liver into the blood, iii) decrease in clearance of triglycerides from plasma. There was no significant difference in hepatic triglyceride content between the treated and control animals. Therefore, the accumulation of hepatic triglyceride and consecutive release from the liver into the blood may not be cited as the cause of hypertriglyceridemia. The decreased removal of triglycerides due to the inhibition of lipoprotein lipase activity in the endothelium of capillaries was not proved to be competent in the hypertriglyceridemic rabbits, irrespective of the slight decrease in plasma lipoprotein lipase activity.

Dingle et al\(^2\)\(^2\) have shown that cobalt ions in vitro inhibit the oxidation of pyruvate and \(\alpha\)-ketoglutarate by mitochondrial suspensions of rat liver and thigh muscle. Wiberg et al\(^8\) have shown that the myocardium of rats treated with cobalt is unable to oxidize pyruvate or fatty acid. The accumulated pyruvate is reduced to lactate. Accumulated pyruvate and lactate can then proceed to glycogen via the established pathways, and fatty acids can be converted to triglyceride.\(^2\)\(^3\) McDermott et al\(^1\)\(^9\) found a remarkably high concentration of plasma lactate among 13 patients, who suffered from myocardial failure due to heavy ingestion of beer and had been admitted to Omaha Veterans Administration Hospital. About a twice increase in lactate concentration in the serum of cobalt chloride treated rabbits suggests that cobalt induces an inhibition of oxidative decarboxylation of pyruvate, which is reduced to lactate in the tissues and results in an increased release of the latter from tissue to blood. In the cobalt sulfate treated rats, on the contrary, no significant increase was found in blood lactate concentration, but the rise in serum pyruvate concentration was significant. Whether the discrepancy between rabbit and rat in the change of lactate or pyruvate concentration can be attributed to species difference, the difference of cobalt compound, or to the amount of administered material is not known.

McDermott et al\(^1\)\(^2\) reported remarkable elevations of serum GOT and LDH in 13 patients admitted to Omaha Hospital. The remarkable rise in total serum LDH activity, which was seen in the cobalt treated animals suggests that cobalt induces tissue damage. The prominent elevation in the percentage of LDH-5 indicates that anaerobically metabolizing tissues such as skeletal muscle may be impaired, since LDH-5 predominates when metabolism is anaerobic. Thorling et al\(^2\)\(^4\) reported that the isozyme pattern of kidney cortex of the rabbits injected with cobalt revealed an increase in the relative content of the \(M\) subunit, which constitutes LDH-5. They postulated that cobalt interferes with certain oxidation enzymes in the cell, in which anaerobic metabolism with high glycolysis is predominant, and consequently the LDH-5 is liberated therefrom into the blood stream.

**SUMMARY**

Cobalt chloride injection (25 mg/kg of body weight) for 7 days induced an approximately seventeen fold increase in the plasma triglyceride of rabbits. The level of plasma FFA, the content of FFA and the activity of hormone sensitive lipase in perirenal adipose tissue, however, were within normal limits. The content of triglyceride in the liver and kidney, as well as the post-heparin lipoprotein lipase activity in plasma also remained within normal range.

Serum LDH activity in cobalt treated rabbits was elevated more than three times compared
with that of control rabbits. The isozyme pattern of LDH revealed a remarkable rise in LDH-5 and a fall of LDH-1.

Blood lactate concentration was elevated significantly, but serum pyruvate remained unchanged in the cobalt chloride treated rabbits. On the contrary, serum pyruvate concentration rose significantly, but blood lactate remained within normal range in rats, which were injected intraperitoneally with cobalt sulfate (4 mg Co**+/kg of body weight) for 8 days.

ACKNOWLEDGEMENT

The authors wish to express their thanks to Miss A. Takano and to Miss Y. Nagao for the technical assistance.

REFERENCES

コバルト塩注射に伴う実験動物の Hypertriglyceridemia
と血清 LDH-アイソサイムの変化

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浅野聡平・駒谷美智子・小池重夫

1963〜66年に欧米諸国の数社のビール工場で、泡の安定剤として、コバルトを加えたところ、大量のビール製造時に致命的な高い心臓疾患が発生して大きな問題となった。塩化コバルトを家兎に注射していると、心臓疾患とともに脂血を起こして来る事は既に報告されている。しかし、如何なる機転で脂血が起こってくるかは不明である。われわれはコバルト注射に伴う脂血、就中 Hypertriglyceridemia の成因をさぐるため、脂質代謝、血清 LDH の面から追究した。

体重 3kg 以上のオスの家兎に 7 日間に亘って 10% の CoCl₂・6H₂O を 25mg/kg 体重の割合で毎日筋肉内に注射し 8 日間に殺した。オスのラットには 4mgCo⁺⁺/kg 体重の割合で CuSO₄・7H₂O を 8 日間に亘って腹腔内に注射した。

塩化コバルト注射家兎の血漿 Triglyceride は対照に比べ約 17 倍も増加した。しかし、血漿 FFA, 腸周囲の脂肪組織の FFA および Hormone sensitive lipase の活性には有意差はみられなかった。また肝, 腎の Triglyceride 量も対照に比べて差がなかった。このことは, 塩化コバルト注射 8 日後の時点で Hypertriglyceridemia の成立を, コバルトによって脂肪組織の Hormone sensitive lipase の活性が高まり, 脂肪組織の FFA が増量して血流中に FFA が増動され, 肝で triglyceride の合成が亢進し, 流血中の triglyceride の増加を来たすという経路に求めることは出来ないことを示唆している。また血流中の Post-heparin lipoprotein lipase の活性は例数が少ないが有意に低下していたなかった。したがって Lipoprotein lipase の活性阻害に Hypertriglyceridemia の成因を求めることもむずかしい。

塩化コバルト注射家兎の血清 LDH の活性は対照に比べて約 3 倍上昇し, LDH の Isozyme は LDH-1 の相対的低下と LDH-5 の上昇が顕著である。また塩化コバルト注射家兎の血中乳酸は有意に増加したが血液ビルピン酸には変化がみられなかった。LDH-5 の上昇と血中乳酸の増加はコバルトによる anaerobic 代謝が亢進していることを物語るものといえよう。

これに反して硫酸コバルト注射のラットでは血漿ビルピン酸が増加した。血中乳酸には増加の傾向がみられたが、対照に比べて有意差はなかった。

（受付1970年10月20日）