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Effect of L-Thyroxine on Some Blood Parameters of Laboratory Mice

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Abstract. The effect of intraperitoneal administration of low dose (1 μg/g) and high dose (4 μg/g) of thyroid hormone after 6,24,72 and 96h on serum proteins, uric acid, triglycerides, glucose and cholesterol in laboratory mice has been investigated. Thyroxine treatment resulted in a biphasic effect on total proteins; lower dose induced anabolic effect while the higher was found to be catabolic. There was an initial decrease in uric acid concentration followed by a significant increase at 24h post-injection. Serum triglycerides and cholesterol values were significantly decreased in the experimental groups. The effect on triglycerides was found to be dose dependent. Both doses of T_4 resulted in a significant decrease in serum glucose level that was directly proportional to the hormonal dosage. The results are discussed in the light of earlier reports on thyroid hormones and mammalian physiology.

Introduction

The physiological importance of thyroid hormone is well established, in different vertebrate groups. In birds and mammals thyroidectomy or hypothyroidism results in general body growth retardation by decreasing the protein and RNA content of skeletal muscle [1 - 4] whereas thyroxine administration increases protein synthesis in muscle [5-10].

In majority of the previous reports [10-12] regarding the role of thyroid hormones in mammalian physiology, muscle and liver have been studied in detail. On the other hand, studies on biochemical and physiological consequences of thyroid treatment are few [13; 14].

Circulating blood is a constant source of supplying and collecting different metabolites from various body tissues, and organs. A study of various metabolites in blood under the influence of thyroid hormone will contribute more to the understanding of its biochemical and physiological effects in the body as a whole and at the tissue level as well. The present communication deals with the effect of a single ip injection of T_4 with time on serum, albumin, cholesterol, glucose, triglycerides and uric acid in mice.

Materials and Methods

Male Swiss albino mice (SWR, home bred) aged 5-6 weeks, weighing 20-30g were obtained from the animal care center, College of Pharmacy, King Saud University, Riyadh. The animals were randomly distributed into three groups of 16 each and maintained at 24±1°C and a photoperiod of 12 L: 12 D. For daylight fluorescent tubes were employed. The mice were acclimatised for eight days in the laboratory and after that allocation of groups to controls and experimentals were made on random basis. During the acclimatisation, as well as the experimental period animals were fed with purina chow diet and water *ad libitum*.

The mice were fasted for 2h before the start of the experiment. Thyroxine (T_4) (Merck, Germany) as its sodium salt was dissolved in alkaline 50% propylene glycol. The hormone was injected intraperitoneally to the individually weighed animals at a dosage of 1.0 and 4.0 μg/g body weight. Controls were injected with carrier only. The doses of T_4 in the present studies were determined from the previous reports on rodents. Hormonal dilutions were adjusted in such a manner that the injection volume remained uniform in all the groups. Four mice were sampled from each group for biochemical analyses at 6, 24, 72 and 96h after a single injection of T_4.

Blood samples were collected from the carotid artery of the anaesthetized dissected animal and allowed to clot and vials containing clotted blood were centrifuged at 5000 rpm for 5 min. to obtain serum. The serum samples exhibiting haemolysis, were discarded. All samples were treated with 0.01 percent chloramphenicol to prevent bacterial growth.

Serum glucose concentrations were measured directly on ASTRA-8, while cholesterol, triglycerides, total proteins, albumin fraction and uric acid were analyzed by COBAS-Bio (Roche). All assays were performed in duplicate. Experimental data were analyzed by Student t-test and graphs were plotted by computer.

Results

The results obtained after injection of 1.0 and 4.0 μg of T_4 on the level of serum metabolites of mice are presented in Figs. 1-3. As seen in Fig. 1 (a,b) both doses of

T_4 resulted in a significant decrease in serum glucose levels after 6h and the decrease was directly proportional to the dose. After 24h, the decrease in serum glucose was still apparent in both experimental groups but it was only statistically significant ($p < 0.01$) in the group which received 1.0 μg/g of T_4 and this trend persisted until 72h after beginning of the experiment. The mice receiving the higher T_4 dose (4.0 μg/g) reflected a significant ($p < 0.001$) hypoglycemic condition compared to controls at 72 h. After 96 h, serum glucose concentrations were comparable in all the three groups.

The effect of T_4 on serum total proteins is presented in Fig. 1 (c,d); 1.0 μg/g elevated, while 4.0 μg/g of T_4 lowered the serum total protein level. Six hours after the single injection of the hormone, serum protein concentrations were significantly higher ($p < 0.01$) in mice administered 1.0 μg/g of T_4, whereas, the group injected with the higher dose of 4.0 μg/g of T_4 showed a significant decrease ($p < 0.01$) compared to controls. After 24h, a comparison of the controls with experimental results revealed that level of serum protein was still higher ($p < 0.05$) in the 1.0 μg/g group while its decreasing trend was more pronounced ($p < 0.001$) in the mice injected with higher hormonal dose. Serum protein concentrations in the two experimental groups were almost comparable to the control values at 72 and 96 h of the experiment. The effect of T_4 on serum cholesterol and triglycerides in mice is plotted in Fig. 2. After six hours of the hormonal treatment, serum cholesterol was significantly depleted in both the experimental groups as compared to controls and a similar trend persisted in both T_4 -treated groups throughout the experimental period, Fig. 2 (a,b). Both doses of T_4 also resulted in significant decline in serum triglycerides and the intensity in decrease remained dose dependent throughout the experiment. In the 1.0 μg/g group concentration of serum triglycerides remained significantly ($p < 0.01$) low as compared to the control values up to 24h after the start of the experiment. After this the decreasing trend of triglycerides was apparent but not statistically significant as compared to the controls. On the other hand, serum triglycerides of mice treated with 4.0 μg/g dose of T_4 remained significantly lower than those of the controls throughout the experiment. The depleting effect of T_4 on triglycerides level was found to decrease with time; the difference from controls being higher at 6h ($P < 0.001$) than at the time of termination of the experiment ($P < 0.05$), Fig. 2 (c,d.) Figure 3 reveals the effect of single ip injection of T_4 on serum albumin and uric acid concentration in mice. Serum albumin levels were found to be significantly elevated in both the experimental groups as compared to controls. This increase in albumin levels was apparent at 6 h and persisted until after 72h of the experiment, Fig. 3 (a,b). On the other hand, serum uric acid concentrations were initially decreased followed by an increase in both the experimental groups. Six hours after the start of the experiment, serum uric acid concentrations were significantly ($P < 0.05$) decreased in the 1.0 μg/g treated group but the decrease was not significant in the mice receiving the higher dose of T_4. After 24h serum levels of uric acid were found to be significantly ($p < 0.001$) increased in both the experimental groups after which time the values in all the three groups were comparable, Fig. 3 (c,d).

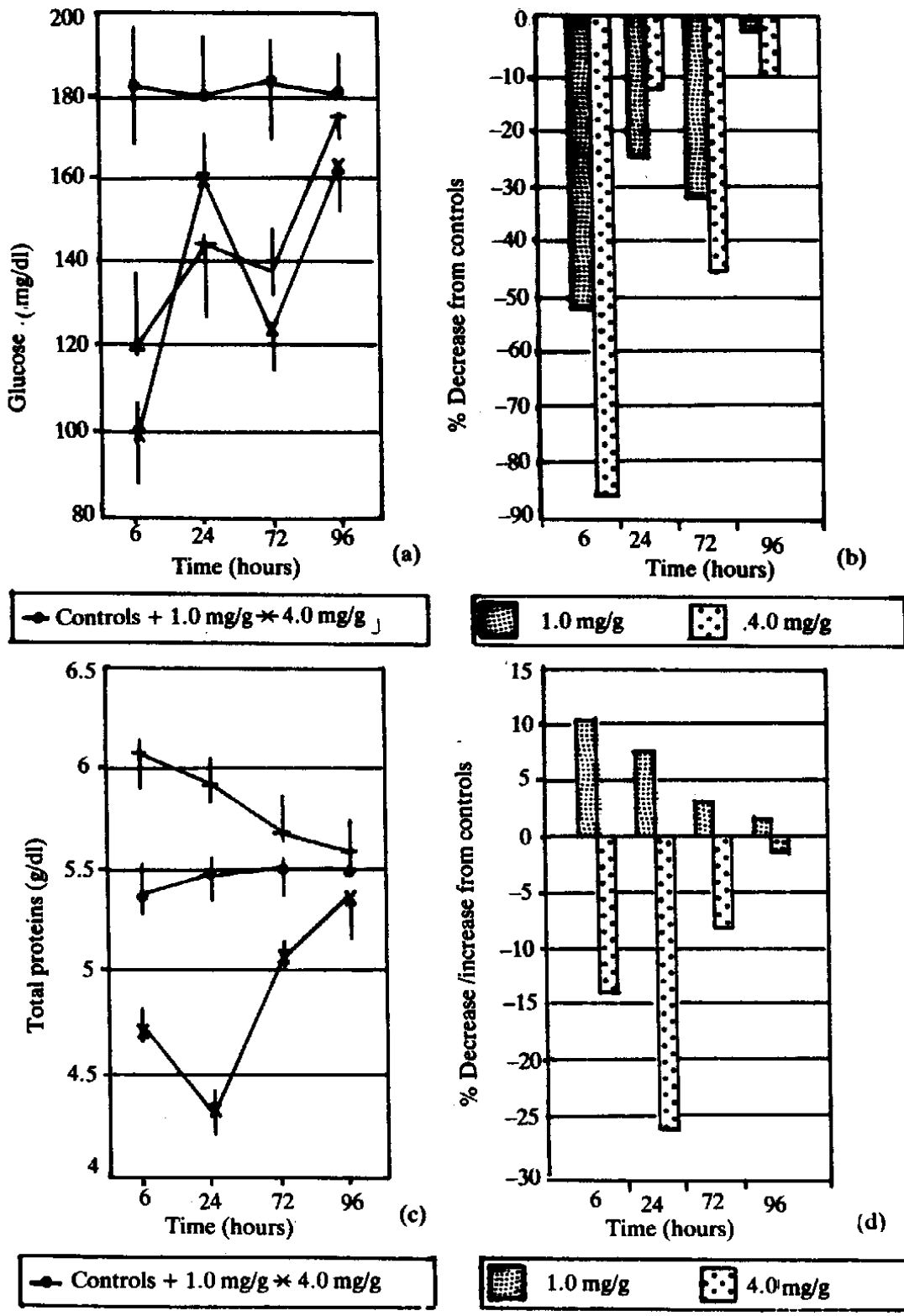
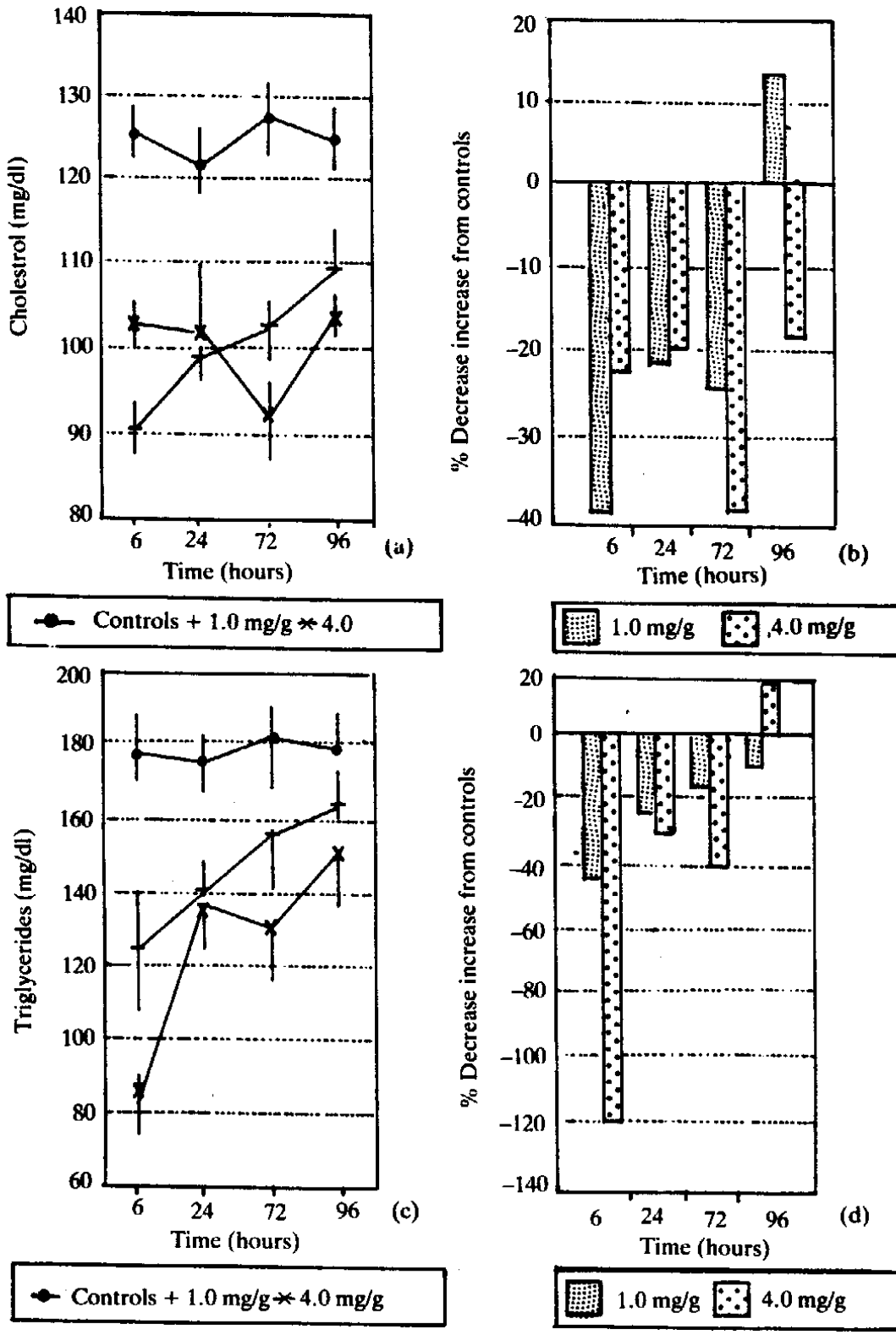


Fig. 1. Effect of single i.p. injection of thyroxin on the conc. of plasma glucose (a,b) & total proteins (c,d).



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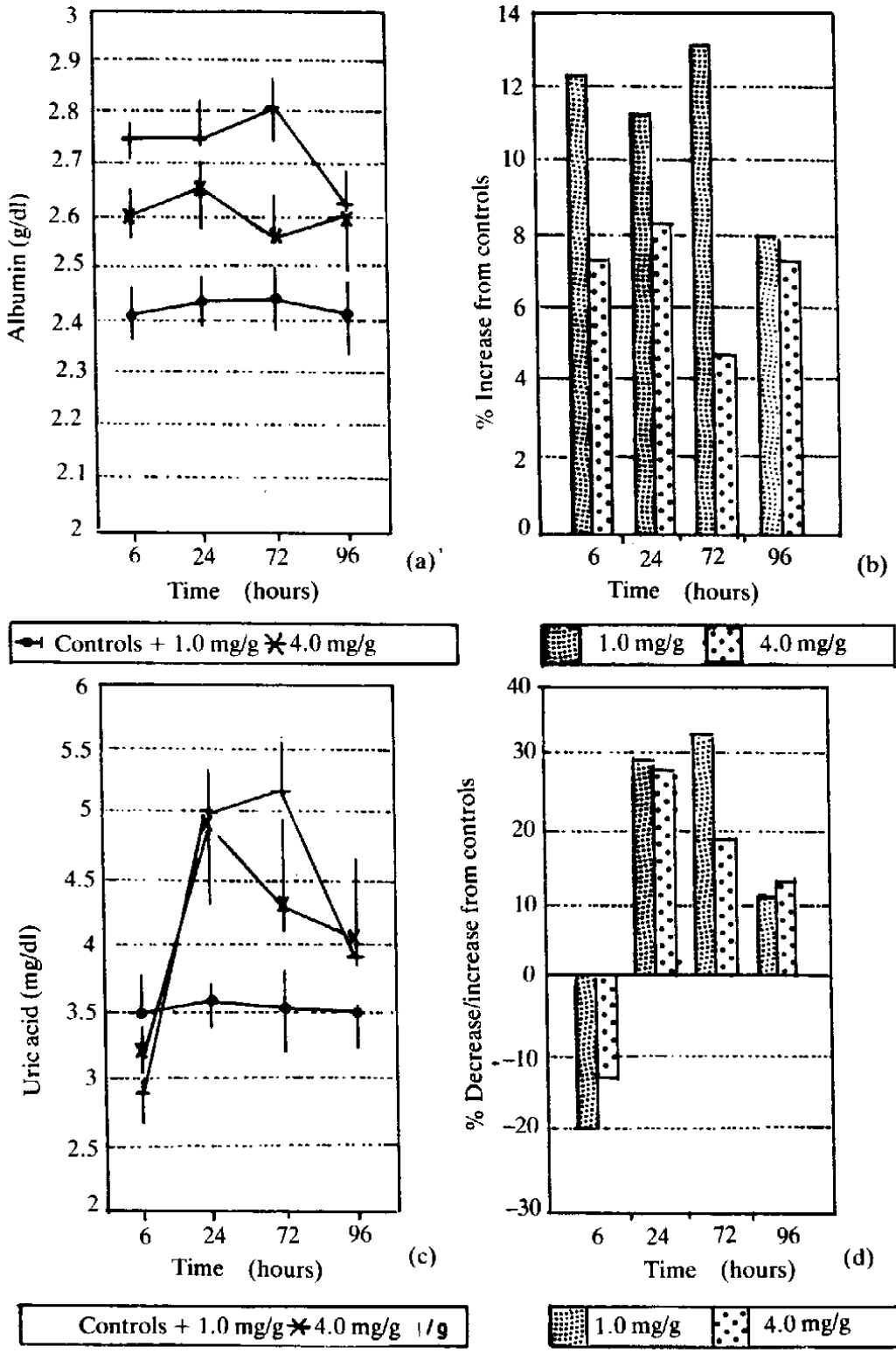

Fig. 3. Effect of single i.p. injection of thyroxin on the conc. of plasma albumin (a,b) & uric acid (c,d).

Discussion

The present study indicates that T_4 is capable of inducing changes in serum glucose, cholesterol, triglycerides, total proteins, albumin fraction and uric acid in male albino mice. A single injection of T_4 (1.0 μg/g) resulted in a significant increase in serum total proteins and albumin after 6h and the significant increase was sustained until after 24h. Higher hormonal dose (4.0 μg/g) induced catabolism in total proteins. Serum levels of glucose, triglycerides and cholesterol were significantly reduced after T_4 administration. Decrease in these parameters was apparent after 6h and continued until 72h, except serum cholesterol values which remained significantly lower than the controls even at the time of termination of the experiment. T_4 treatment resulted in an initial decrease in serum uric acid levels followed by a significant increase after 24h post-injection time.

The role of thyroid hormones in mammalian growth is well established [15, 16a, 16b]. Effects of T_4 on cellular actions and bone mineral density have been evaluated in some detail [7, 11, 14, 17-19]. The studies indicate that thyroid hormone action at cellular level is mediated through the active synthesis of both messenger RNA and ribosomal RNA by activation of RNA polymerase I and II. The results of the present study indicate that T_4 induces similar physiological changes in blood as have already been reported for the mammalian liver and muscle. Our present findings are further supported by a recent study [20] in rats; experimental induction of hypothyroidism by carbon tetrachloride resulted in a significant decrease in serum total proteins. In the present study, however, a higher dose (4.0 μg/g) resulted in a catabolic effect on total serum proteins (Fig. 1-c). The mechanism(s) of this opposite effect of T_4 in the same animal and tissue is not known. It may, however, be mentioned that protein level in a cell depends upon both, the rate of synthesis as well as on the rate of their degradation. On higher T_4 dose it is possible that the metabolic process in the cell increases and the degradation rate exceeds the rate of protein synthesis [21]. The above hypothesis is also supported by our present findings on serum uric acid concentrations. Uric acid is the end product of protein metabolism and a decrease in serum total proteins, after 6h in the 4.0 μg/g group, was followed by a significantly elevated uric acid concentrations at 24h. Seventy-two hours after the experiment, a recovery in serum proteins was followed by normal uric acid level. Moreover, it has been established [22] that pharmacological hyperthyrodism is accomplished by a corresponding increase in lysosomal enzyme cathepsin-D, which degrades the proteins in different tissues.

T_4 administration significantly lowered serum glucose in both the experimental groups after 6h and this effect persisted until 72h. Hypoglycemia observed in the experimental animals was also accompanied by a significant decrease in serum triglyceride and cholesterol values. Our observation of reduced serum cholesterol levels in the experimental animals is in agreement with the report [23] where

hypothyroidism was accompanied by elevated serum cholesterol levels in humans. These lipids are highly concentrated stores of metabolic energy [24] and it appears that under the influence of T_4, lipids are being mobilised to meet the metabolic demands and proteins are being saved for growth. Administration of low doses of thyroid hormones are known to increase the protein synthesis and induce body growth [16a; 18; 20; 21; 25] and are also known to regulate insulin-like growth factor-I (IGF-I) expression directly as well as by cooperation with endogeneous growth hormone [16 b]. Hypoglycemic conditions observed during most of the experimental period in T_4 treated animals could have developed either due to an increased insulin level under the influence of expected hyperglycemia as a result of lipolysis or due to direct stimulation of endocrine pancrease by increased T_4 levels or both. In fact, plasma insulin levels have been reported [13; 15; 26] to be significantly elevated in hyperthyroid pigeons, rats and mice.

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تأثير الثيروكسين على بعض المؤثرات في دم فئران التجارب

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(سُلِّمَ في ٢٤/رمضان/١٤١٣هـ، وقُبل للنشر في ١٦/جمادى الثانية/١٤١٤هـ)

ملخص البحث. لقد دُرس تأثير حقن جرعات منخفضة (١ ميكروجرام/جرام) وجرعات عالية (٤ ميكروجرام/جرام) من هرمون الثيروكسين في التجويف البطني لفئران التجارب بعد ٦، ٢٤، ٧٢ و٩٦ ساعة على البروتين، حامض اليوريك، الجلسرويد الثلاثي والكولسترول في دم فئران التجارب. وجد أن تأثير هرمون الثيروكسين على البروتين الكلي عندما يعطي بهذه الطريقة يكون تأثيرًا بنائيًا عندما تكون الجرعة منخفضة ويكون التأثير هدميًا عندما تكون الجرعة عالية. كان هناك انخفاض مبدئي في تركيز حامض اليوريك تُبِعَ بارتفاع معنوي بعد مرور ٢٤ ساعة على الحقن. كان هناك انخفاض معنوي في قيمة كل من الكولسترول والجلسرويد الثلاثي في دم الحيوانات التي حُقِنَتْ بالثيروكسين. ووجد أن التأثير على الجلسرويد الثلاثي يعتمد على تركيز الثيروكسين خلال فترة التجربة.

وقد نوقشت النتائج على ضوء الدراسات السابقة على هرمون الثيروكسين وفسيولوجيا الثديّات.