levels in chronic alcoholics demonstrated the expected common occurrence of deficiencies in each, but failed to show a correlation of these parameters in the individual alcoholic patients.

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Received July 11, 1966. P.S.E.B.M., 1966, v123.

## Induction of Mitochondrial α-Glycerophosphate Dehydrogenase by Thyroid Hormone: Effect of Fasting and Refeeding.* (31575)

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Previous studies have shown that the induction of mitochondrial L-α-glycerophosphate dehydrogenase in rat liver (LM-GPDH) by triiodothyronine ($T_3$) may result from the acceleration of enzyme protein synthesis as judged by the fact that this induction is sensitive to inhibition by ethionine, puromycin, actinomcyin D, and 5-fluorouracil(1, 2). Starvation has been shown to suppress protein synthesis(3-5) and also to block the increase of basal metabolic rate and growth caused by $T_3$-administration(6). Therefore, the effect of fasting and refeeding on the induction of LM-GPDH by $T_3$-administration was investigated.

*Materials and methods.* The rats utilized, weighing between 200 and 280 g, obtained from Charles River Laboratories, were fed a control synthetic diet with the following composition: casein, 18%; dextrose, 72%; corn oil, 5%; salt-mixture (4%) and a vitamin-mixture (1%) which contained all of the known required vitamins and minerals(7). Rats were not used until they had eaten this diet for at least 5 days. The diets used in refeeding the animals after fasting contained different proportions of carbohydrate, protein and/or fat. Salt-mixture (5%) was always added to each diet used for refeeding but the vitamin-mixture was omitted owing to the brevity of the feeding period.

3,3',5-L-triiodothyronine sodium salt, ob-

* Supported by USPHS grant TIGM-648-05. The experimental data are taken from a dissertation submitted by Kai-Lin Lee to the Graduate School of Tulane University in partial fulfillment of the requirements for the degree of Doctor of Philosophy 1966.

tained from Sigma Chemical Co., was prepared in alkaline-saline(8) and injected intraperitoneally at a dosage of 100 μg per 100 g of body weight. The mitochondria were isolated from liver homogenates made in 0.25 M sucrose according to the procedure of de Duve *et al*(9). The mitochondrial pellet was resuspended in 0.25 M sucrose and washed twice and finally suspended in 0.05 M sodium phosphate buffer, pH 7.6 at a concentration of mitochondria equivalent to 0.06 to 0.15 g of fresh liver per ml. Twenty to twenty-five μl of this suspension was used to assay for LM-GPDH activity which was determined by the method described by Y-P. Lee and Lardy(10), with slight modifications (11). One unit of LM-GPDH activity was defined as 0.001 change in optical density per minute determined at 500 mμ. Mitochondrial protein was determined according to the method of Lowry *et al*(12). DNA was isolated from the liver homogenates by the method of Schneider(13) and quantitatively determined by using diphenylamine reagent(14).

*Results and discussion.* Since extensive changes in chemical composition of liver which result during fasting(15) have been observed, various parameters were chosen to evaluate the behavior of LM-GPDH activity during fasting. The body weights of all rats, used in this particular experiment, were standardized between 258 to 262 g. It is evident from the data shown in Table I that the enzyme activity gradually decreased during fasting when the enzyme activity was calculated based on body weight (units per 100 g), total activity in liver (units per liver), or DNA (units per mg). However, the LM-GPDH activity was essentially unchanged during fasting if mitochondrial protein was chosen as the base-line parameter (units per mg protein). These data may result from the fact that, under fasting conditions, the decrease of enzyme protein is parallel to the decrease of total mitochondrial protein. Total mitochondrial protein per liver indeed decreased in a fashion similar to the enzyme activity per liver (Table I).

The results shown in Table II demonstrate that the induction of LM-GPDH is greatly suppressed by starvation. When $T_3$ was given

TABLE I. Effect of Fasting on LM-GPDH Activity.

| Fasting (days) | Avg body wt (g) | | Avg liver wt (g) | Mitochondrial protein (mg/liver) | No. of rats | Units of LM-GPDH activity × $10^{-3}$ per* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Initial | Final | | | | 100 g body wt | Liver | mg mitochondrial protein | DNA |
| 0 | 261 | 261 | 10.8 | 421 | 5 | 23.5 ± .30 | 61.1 ± .85 | .145 ± .002 | 2.01 ± .043 |
| 1 | 260 | 240 | 6.6 | 315 | 4 | 23.2 ± .67 | 54.2 ± 2.97 | .149 ± .004 | 1.79 ± .044 |
| 2 | 260 | 224 | 6.6 | 289 | 4 | 19.4 ± .55 | 43.4 ± 1.57 | .149 ± .003 | 1.45 ± .015 |
| 3 | 260 | 214 | 6.2 | 241 | 5 | 14.7 ± .32 | 31.5 ± .84 | .133 ± .003 | 1.16 ± .030 |
| 4 | 260 | 199 | 5.6 | 201 | 6 | 13.1 ± .32 | 26.1 ± .62 | .130 ± .008 | .95 ± .074 |
| 5 | 260 | 185 | 4.8 | 184 | 6 | 12.1 ± .45 | 23.1 ± .85 | .130 ± .004 | .84 ± .028 |
| 6 | 262 | 180 | 4.6 | 172 | 6 | 11.1 ± .27 | 22.2 ± 1.01 | .125 ± .006 | .80 ± .018 |

* Mean ± standard error of mean.

to the 4-day fasted rats, LM-GPDH activity increased by only about 60% during a 24-hour period after $T_3$ administration; however, under the identical experimental conditions $T_3$ caused a 150% increase of LM-GPDH activity in well-fed animals. When food and $T_3$ were given at the same time to 4-day fasted rats, there was a 2-fold in-

TABLE II. Effect of Fasting and Refeeding in Induction of LM-GPDH by $T_3$.

| Nutritional status† | No. of rats | Units of LM-GPDH activity × $10^{-3}$ per* 100 g body wt | mg mitochondrial protein |
|---|---|---|---|
| Well-fed | 4 | 19.2 ± .29 | .137 ± .007 |
| Well-fed + $T_3$ | 6 | 41.3 ± .74 | .347 ± .014 |
| 5-day-fasted | 6 | 10.4 ± .96 | .134 ± .015 |
| 5-day-fasted + $T_3$ | 5 | 16.1 ± .59 | .218 ± .008 |
| 4-day-fasted and one-day-refeeding | 6 | 20.8 ± .14 | .143 ± .004 |
| 4-day-fasted and one-day-refeeding + $T_3$ | 6 | 43.1 ± .59 | .308 ± .011 |

* Mean ± standard error of mean. † For details, see text.

crease in LM-GPDH activity of $T_3$-treated rats over control rats given alkaline-saline rather than $T_3$ (Table II). In other words, one day of refeeding restored about 60% of the reduced induction caused by 4 days of fasting. It is also evident, from the results shown in Table II, that refeeding of 4-day starved rats for one day restored the endogenous enzyme level essentially to normal.

To evaluate further the significance of dietary components used to refeed the starved rats, in order to restore the LM-GPDH induction and the endogenous enzyme level, diets were used which contained different proportions of carbohydrate, fat and protein. In these experiments enzyme activity based on body weight (units per 100 g) before sacrifice was used to evaluate the endogenous enzyme level while specific activity based on mitochondrial protein (units per mg) was the parameter used to evaluate the induction. The results shown in Table III were obtained when 2 dietary components were used to refeed 4-day fasted animals. Comparing with the results of saline-treated control animals it is evident that either a diet containing protein and carbohydrate or protein and fat essentially restores the endogenous enzyme level; however, a carbohydrate and fat diet poorly restored the endogenous enzyme level. These results indicate that protein may be essential in restoring the endogenous enzyme level. However, in evaluating these diets for the restoration of enzyme-induction, the LM-GPDH activities were increased by 2.2, 1.9, 2.3, 2-fold respectively for the control diet, protein and carbohydrate-diet, fat and carbohydrate-diet and protein and fat-diet. These results indicate that the carbohydrate and fat-diet also is effective in the restoration of enzyme induction. This point was further evaluated by using protein, carbohydrate or fat as a single dietary component to refeed 4-day fasted rats and the results are shown in Table IV. Even though a small number of rats was used in some experiments, there was consistent tendency to show that feeding fat or carbohydrate will restore enzyme-induc-

TABLE III. Effect of Refeeding with Two Dietary Components on Restoration of Induction of LM-GPDH by $T_3$ and Its Endogenous Level of 4-Day Starved Rats.

| Diet | Treatment | No. of rats | Avg body wt (g) † | Avg liver wt (g) | Mitochondrial protein (mg/liver) | Units of LM-GPDH activity × $10^{-3}$ per* 100 g body wt | mg mitochondrial protein |
|---|---|---|---|---|---|---|---|
| Carbohydrate, 65; protein, 25; fat, 5 | Saline | 4 | 175 | 9.6 | 285 | 20.8 ± .14 | .143 ± .004 |
| | $T_3$ | 4 | 182 | 7.3 | 356 | 41.3 ± .59 | .308 ± .011 |
| Carbohydrate, 70; protein, 25 | Saline | 3 | 186 | 9.3 | 239 | 17.9 ± .73 | .150 ± .008 |
| | $T_3$ | 4 | 182 | 7.2 | 328 | 38.3 ± .15 | .298 ± .008 |
| Carbohydrate, 70; fat, 25 | Saline | 3 | 177 | 6.0 | 263 | 14.1 ± .36 | .161 ± .012 |
| | $T_3$ | 5 | 174 | 5.1 | 300 | 32.1 ± .90 | .367 ± .012 |
| Protein, 25; fat, 70 | Saline | 3 | 177 | 6.6 | 341 | 19.6 ±1.15 | .153 ± .004 |
| | $T_3$ | 6 | 175 | 6.4 | 364 | 38.9 ± .98 | .299 ± .003 |

* Mean ± standard error of mean. † After 4 days of fasting, for other details see text.

TABLE IV. Effect of Refeeding with a Single Dietary Component on Restoration of Induction of LM-GPDH by $T_3$ and Its Endogenous Level of 4-Day Starved Rats.

| | | Units of LM-GPDH activity × $10^{-3}$ | | | |
|---|---|---|---|---|---|
| | | 100 g body wt | | mg mitochondrial protein | |
| Diet | Treatment† | 24 hr | 48 hr | 24 hr | 48 hr |
| Carbohydrate, 65; protein, 25; fat, 5 | Saline | 18.6 ± .90 (6)* | 17.5 ± 1.97 (4) | .141 ± .003 | .140 ± .021 |
| | $T_3$ | 37.6 ± 1.55 (6) | 50.1 ± 1.23 (4) | .297 ± .012 | .451 ± .013 |
| Carbohydrate | Saline | 11.8 ± .26 (4) | 12.9 ± 1.47 (4) | .144 ± .006 | .156 ± .009 |
| | $T_3$ | 21.6 ± 1.08 (6) | 37.9 ± 2.02 (5) | .305 ± .006 | .626 ± .028 |
| Protein | Saline | 14.0 ± 1.05 (4) | 17.6 ± 2.84 (3) | .113 ± .009 | .130 ± .006 |
| | $T_3$ | 20.0 ± 1.07 (5) | 25.2 ± 2.00 (4) | .170 ± .008 | .232 ± .008 |
| Fat‡ | Saline | 11.8 ± .76 (3) | 11.9 ± .78 (4) | .130 ± .003 | .140 ± .015 |
| | $T_3$ | 26.3 ± 1.19 (4) | 51.0 ± 2.34 (4) | .297 ± .007 | .699 ± .027 |

* Mean ± standard error of mean. Numbers in parentheses represent No. of animals per group.

† For details see text.

‡ Crisco oil was used instead of corn oil.

tion under the influence of $T_3$, while the protein is comparatively poor in this regard. This observation was especially evident when rats were sacrificed 48 hours after $T_3$-administration and refeeding. The different effectiveness of the dietary components in restoring $T_3$-induced LM-GPDH activity conceivably might result from substrate induction, owing to the fact that fat and carbohydrate are good sources of glycerophosphate. However, this possibility is unlikely because all attempts to show substrate induction for this enzyme have given essentially negative results(11). Alternately these effective differences may be related to the energy they provide. This suggestion is strengthened by the observation that 4-day fasted rats, when refed with fat, exhibited better $T_3$-induction than rats refed with carbohydrate.

The present observations are consistent with the view that the diminution of $T_3$-induction after fasting may result primarily from an energy-deficiency. Preliminary results have also revealed that $T_3$ fully induces LM-GPDH in rats which have eaten a protein-free diet for 9 days, which further indicates that $T_3$-induced LM-GPDH synthesis is not dependent on the dietary protein.

*Summary.* Starvation was found to decrease both the endogenous LM-GPDH level and the induction of LM-GPDH by $T_3$-treatment. Different diets of protein, carbohydrate, or fat along with a salt-mixture were used to refeed 4-day fasted rats; it was found that protein, required for restoration of endogenous enzyme levels, did poorly restore $T_3$-induction; however, either fat or carbohydrate, which poorly restored endogenous enzyme level, did restore $T_3$-induction. These observations suggest that $T_3$-induced LM-GPDH synthesis is independent of the dietary protein and that the diminution of $T_3$-induction after fasting may primarily result from energy deficiency.

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Received July 13, 1966. P.S.E.B.M., 1966, v123.

# Effect of High Lipid Diets on Normal and Traumatized Rats.* (31576)

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Diets rich in saturated fat are reported to diminish blood proteolytic activity in a variety of mammals(1-9). Such a depression could enhance blood coagulability by inhibiting A) the blood proteolytic enzyme, plasmin; B) the activation of its precursor, plasminogen; or C) normal maintenance of blood plasminogen levels. In seemingly unrelated studies, Duguid(10) has reported that thrombi lodged at the intimal surface of arteries may readily become endothelialized and undergo necrotic conversion into atheromatous plaques. These findings suggest that lipemia might induce the development of atherosclerosis by depressing proteolytic activity and thereby enhance the production of thrombi.

In this study, direct inhibition of plasmin activity by lipemia, particularly hypercholesterolemia, was investigated by measuring serum proteolytic activities in rats maintained on diets rich in a highly saturated fat (butter), a relatively unsaturated fat (corn oil) and a normal diet containing lipid levels of 1/10 the experimental diets.

Plasminogen reserves in the lipemic and normal rats were assessed using trauma as the means of plasminogen activation. Rats from each diet category were subjected to doses of trauma in a Noble-Collip Drum shown to yield 2- and 4-fold increases in the plasmin levels of normal rats(11). If trauma failed to activate similar plasmin levels in lipemic rats one would assume that lipemia inhibited plasminogen activation or in some way depressed body plasminogen concentrations.

*Methods.* Ninety male Holtzman rats were maintained on one of the following experimental diets: A) 40% whole butter diet, B) 40% corn oil diet and C) normal diet of Wayne Lab Blox containing 4.9% lipid by weight.

These diets were compounded as follows:

| 40% lipid diet[1] | g % |
|---|---|
| Cholesterol | 5.0 |
| Choline chloride | .2 |
| Sodium cholate | 2.0 |
| Salt mixture[2] | 4.0 |
| Vitamin mixture[3] | 2.0 |
| Casein | 20.0 |
| Sucrose | 26.8 |
| Whole butter or corn oil | 40.0 |
| **Normal diet[4]** | |
| Crude protein (min) | 26.0 |
| Crude fat | 4.9 |
| Crude fiber (max) | 6.5 |
| Crude ash (min) | 7.5 |

[1] Purchased from Nutritional Biochemicals Corp. —approximately 610 calories/100 g.

[2] Salt mixture U.S.P. XIV from N.B.C.

| | g | |
|---|---|---|
| Cupric sulfate | 0.48 | 16.2 g |
| Ferric ammonium citrate | 94.33 | |
| Manganese sulfate | 1.24 | |
| Ammonium alum. | 0.57 | |
| Potassium iodide | 0.25 | |
| Sodium fluoride | 3.13 | |
| to make | 100.00 | |
| Calcium carbonate | 68.6 | |
| " citrate | 308.3 | |
| " biphosphate | 112.8 | |
| Magnesium carbonate | 35.2 | |
| " sulfate | 38.3 | |
| Potassium chloride | 124.7 | |
| Dibasic potassium phosphate | 218.8 | |
| Sodium chloride | 77.1 | |
| | 1000.00 | |

[3] Vitamin fortification mixture from N.B.C. without choline chloride.

* This study was supported by a grant from Nat. Inst. Health, Grant H-3307.

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