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Effect of Hydrazine on Protein Content of Various Tissues in the Rat.* (30425)

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Hepatotoxic effects of hydrazine on lipid and carbohydrate metabolism have been established both biochemically and histologically. Fatty lesions in the liver and kidney have been reported after exposure of various laboratory animals to hydrazine(1,2,3). Underhill(4) noted a decrease of glycogen stores in the liver and muscle following administration of hydrazine to dogs. More recently, lipid accumulation in the liver has been correlated with the reduction in liver glycogen of hydrazine-treated rats(5).

Although such profound alterations of carbohydrate and lipid metabolism have been observed, the effects of hydrazine on protein metabolism have not been explored thoroughly. Louis and Lewis reported that the percentage of nitrogen and sulfur of the heat-coagulable protein in rabbit liver was unaltered by inanition and hydrazine treatment (6). However, these authors did not measure the protein content of the liver.

Amenta and Johnston(7) observed a stimulation of the incorporation of labelled amino acids into protein in liver slices from hydrazine-treated rats as compared to untreated controls. They attributed the increased incorporation of amino acids into protein to a rise in the amino acid pool size brought about by a blockage in conversion of amino acids to keto acids in the liver cell. However, an elevation in the amino acid pool size would be expected to result in a diminished incorporation of the labelled molecules into protein. Thus, an alternate explanation, that of increased protein biosynthesis, was explored.

Hydrazine may affect protein metabolism *in vivo* by alteration of cellular metabolic pathways as well as by alteration in the nutritional state of the animal, since considerably reduced food intakes have been demonstrated following hydrazine treatment(5). To evaluate the effect of hydrazine on the protein metabolic pathways only, it is necessary to compare hydrazine-treated animals to non-treated animals that have been deprived of food over the same period of time, since fasting alone has been shown to produce marked changes in protein biosynthesis in various tissues(8).

Methods. Three groups of 10 adult male rats of the Sprague-Dawley strain were treated as follows: a fed-control group was killed at start of the experiment; the fasted group was deprived of food for 24 hours and killed; the hydrazine group was injected in-

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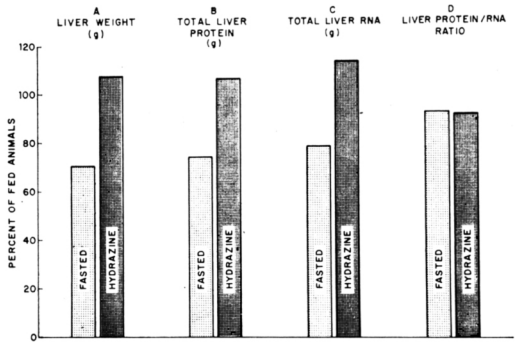

FIG. 1. Effect of hydrazine treatment and of fasting on liver weight, total liver protein, total liver RNA, and liver protein/RNA.

The results for the hydrazine and fasted groups are represented as % of levels for the fed animals. Bars illustrated for the hydrazine and fasted animals are significantly different ($p<.01$) in A, B, and C. (Data from Tables I and II may be used to reconstruct this figure).

traperitoneally with 40 mg/kg of hydrazine (brought to pH of 7.4 with CO_2), fasted for 24 hours and killed. Water was available *ad libitum* throughout the experiment.

All animals were anesthetized with sodium pentobarbital (50 mg/kg), killed by exsanguination and the liver, kidney and gonads were removed and weighed. Each individual tissue was homogenized in distilled water with a water-jacketed, ground-glass, Potter-Elvehjem homogenizer.

Total serum protein concentrations were estimated by the biuret method of Layne(9). Protein and RNA content in duplicate aliquots from each tissue homogenate were determined by a modification of the procedure of Wannemacher *et al*(10).

An analysis of variance was performed on the means of the duplicate determinations and .01 was taken as the level of significance. Variances were calculated for each of the 3 treatment groups. These variances were pooled and used to calculate the standard deviations which are recorded in the Tables.

Results and discussion. The livers from the hydrazine-treated animals were significantly larger than those from the fasted rats after 24 hours (Fig. 1A). Since the mean body weights of these 2 groups were the same at time of autopsy, the relative liver weights (g/100 g body weight) were also greater in the hydrazine-treated than in the fasted group of animals.

Since the total quantity of liver protein (Fig. 1B) was markedly elevated by hydrazine treatment, the enlargement in the liver of the hydrazine-treated animals appears to be associated with a concomitant elevation in liver protein. In addition, a significantly higher level of total liver RNA was observed in the hydrazine-treated groups of animals (Fig. 1C). Yet hydrazine treatment did not alter the concentration of protein (mg protein/g liver) or the concentration of RNA (mg RNA/g liver) in this organ (Tables I and II).

Since the ratio of protein to RNA in the liver was the same for all groups (Fig. 1D), the quantity of protein appeared to be correlated *in vivo* with the quantity of RNA and was independent of the treatment. The constancy of this ratio in the liver of animals in various nutritional states has been reported(11). Furthermore, the constancy of this ratio argues against the possibility that the increased liver protein may be the result of an appreciable pooling of blood in this organ in response to the hydrazine treatment.

The elevation in both RNA and protein suggests that hydrazine has produced a stimulation of protein biosynthesis in the liver. A hydrazine-induced stimulation of protein

TABLE I. Protein Content of Various Tissues.

Treatment	Liver protein conc mg protein / g liver	Total liver protein mg	Kidney protein conc mg protein / g kidney	Total kidney protein mg	Gonadal protein conc mg protein / g gonad	Total gonadal protein mg	Serum protein conc g protein / 100 ml serum
Fed	107.2	1193.0	93.10	186.5	55.92	157.0	5.56
Fasted	112.8	886.1	87.34	203.0	57.62	161.4	5.69
Hydrazine	104.3	1276.0	82.29	202.1	53.74	172.8	5.91
Stand. dev.	9.65	136.0	6.54	25.35	4.76	39.88	.44

TABLE II. RNA Content of Various Tissues.

Treatment	Liver RNA conc mg RNA / g liver	Total liver RNA mg	Kidney RNA conc mg RNA / g kidney	Total kidney RNA mg	Gonadal RNA conc mg RNA / g gonad	Total gonadal RNA mg
Fed	10.35	115.2	6.22	12.56	4.99	14.04
Fasted	11.52	90.79	5.46	13.07	4.97	14.02
Hydrazine	10.74	131.2	5.70	14.29	4.38	14.39
Stand. dev.	.83	13.36	.67	2.47	.57	3.45

biosynthesis in the liver would be consistent with the increased incorporation of labelled amino acids into protein, which has been demonstrated *in vitro*(7).

A significant reduction in liver protein content was found in the fasted animals in comparison to the fed group (Fig. 1B). Since the hydrazine-treated animals were fasted over the 24-hour period, the hydrazine-induced stimulation of protein biosynthesis was sufficient to overcome the reduction in liver protein associated with fasting. Thus, the effect of hydrazine on liver protein appears to be related to metabolic rather than nutritional factors.

Many of the serum proteins are produced by the liver, and any general stimulation of protein biosynthesis by that organ may be reflected by increased serum protein content. However, total serum protein concentration after 24 hours was not altered significantly in these animals by hydrazine treatment (Table I).

Of the organs studied, hydrazine treatment appears to exert an effect on RNA and protein of the liver only. After 24 hours, kidney and gonadal RNA and protein concentrations (Tables I and II), as well as total organ contents of protein and RNA, were the same in the hydrazine-treated and fasted animals. The ratio of kidney and gonadal protein to RNA was also constant, which substantiates the interdependence of these quantities *in vivo*.

Summary. Hydrazine treatment caused an enlargement in livers of rats which appeared to be associated with increased protein content of that organ. This rise in liver protein content was correlated with an increased liver RNA. Similar changes in protein and RNA were not observed in the kidney and gonads. The hydrazine-induced elevation in both liver protein and RNA content is consistent with a stimulation of protein biosynthesis. Furthermore, the effect of hydrazine on liver protein appears to be primarily metabolic rather than nutritional.

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1. Wells, H. G., J. Exp. Med., 1908, v10, 457.
2. Yard, A. S., McKennis, H., J. Pharmacol. Exp. Therap., 1955, v114, 391.
3. Dominguez, A. M., Amenta, J. S., Hill, C. S., Domanski, T. J., Aerospace Med., 1962, v33, 1094.
4. Underhill, F. P., J. Biol. Chem., 1914, v17, 293.
5. Amenta, J. S., Johnston, E. H., Lab. Invest., 1962, v11, 956.
6. Louis, L., Lewis, H. B., J. Biol. Chem., 1944, v153, 381.
7. Amenta, J. S., Johnston, E. H., Lab. Invest., 1963, v12, 921.
8. Allison, J. B., Wannemacher, R. W., Jr., Banks, W. L., Jr., Fed. Proc., 1963, v22, 1126.
9. Layne, E., in Methods in Enzymology 3, Academic Press, Inc., N. Y., 1957, p450.
10. Wannemacher, R. W., Jr., Banks, W. L., Jr., Wunner, W. H., Anal. Biochem., 1965, v11, 320.
11. Banks, W. L., Jr., Allison, J. B., Wannemacher, R. W., Jr., J. Nutrition, 1964, v82, 61.

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