

## THE INFLUENCE OF EPINEPHRINE AND OF STRONG ELECTRICAL STIMULATION ON THE NUCLEIC ACIDS OF RAT LIVER

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**Abstract**—Large doses of epinephrine lead to diminution of RNA contents in the cytoplasmic and mitochondrial fractions of the hepatic cells in rats. Histological analysis showed that epinephrine provoked dystrophic changes in the liver of rats similar to those induced by strong stimulation. Changes in RNA contents are also similar in both cases. The received data exposing the similarity of organic reactions to stimulation and to treatment with epinephrine indicate the significant participation of the sympathetic nervous system and of catecholamines in the development of these neurogenic dystrophies in liver.

As is known from histological investigations, large doses of epinephrine, which reduce mitotic activity, cause reduction in the nucleic acids of the hepatic cells. There are few biochemical studies on the effect of epinephrine on the contents of protein and nucleic acids in the liver and they are not in agreement.<sup>4, 8, 10</sup> There are indications that large doses of epinephrine induce dystrophic changes in various organs including the liver.<sup>9</sup> We have shown in previous investigations that severe stimulation by passage of an alternating electric current through electrodes inserted into the forepaws of immobilized rats for 3 hr causes dystrophic changes in the liver<sup>6</sup>. Pharmacological analysis showed that this hepatic dystrophy is of reflex origin and that the sympathetic nervous system plays a part in its development.<sup>7</sup> It is also known that trophic processes are connected with protein metabolism and disturbance of protein synthesis may be one of the early signs of dystrophy.<sup>1, 2</sup> We have observed subcellular alterations in the hepatic cells, including fragmentation of the ergastoplasm with decrease in the polysomes and ribosomes, as a result of severe stimulation of rats. These alterations are evidently connected with a disturbance of protein and nucleic acid metabolism.<sup>5</sup>

The aim of the present investigation was to determine the nucleic acid contents in different subcellular fractions of the hepatic cell after treatment of rats with epinephrine and at various stages of dystrophy following severe electrical stimulation of the animals.

### METHODS

Male albino rats weighing 190–250 g were fasted for 24 hr before use. Epinephrine was administered as a 0.1% solution in doses of 2.5 ml/kg body wt. by i.p. injection and the animals were killed 15 min later. Rats of the other group were immobilized and subjected to stimulation for 15 min or 3 hr by rectangular impulses of alternating current from a stimulator with 50 Hz frequency, 10 msec. duration and 5–7 V tension on output<sup>3</sup> and then killed immediately. All animals were killed by

decapitation and their livers were taken for determination of the RNA and protein content of whole homogenate and subcellular fractions, including nuclear, mitochondrial and cytoplasmic fractions. The nuclear fraction was sedimented by centrifugation of the homogenate in 0.25 M sucrose at 1000 *g* and the mitochondrial fraction obtained by centrifugation at 10,000 *g* according to Hogeboom, Schneider and Palade.<sup>11</sup> RNA was determined by the method of Rho and Bonner<sup>12</sup> with an 18-hr period of alkaline hydrolysis and subsequent addition of perchloric acid. Total protein was determined by a biuret method after precipitation with trichloroacetic acid and extraction of lipids with an alcohol-ether mixture. The contents of RNA are expressed per mg. protein.

### RESULTS

The effects of epinephrine and of electrical stimulation on the RNA of the hepatic cell were studied and the quantity of RNA in the different subcellular fractions was determined.

Large doses of epinephrine (2.5 ml/kg body wt.) led to diminution of RNA in the cytoplasmic and mitochondrial fractions at 15 min after injection (Table 1). Histological examination showed that dystrophic changes similar to those induced by electrical stimulation appeared in the livers from the rats treated with epinephrine. Groups of cells with pale tinted or imperceptible nuclei were observed in frequent microscopical fields (Fig. 1). These histological observations are in agreement with published data.<sup>9</sup>

TABLE 1. CONTENTS OF RNA IN THE HEPATIC CELL FRACTIONS OF RATS UNDER DIFFERENT EXPERIMENTAL CONDITIONS

Experimental conditions	Quantity of animals	RNA $\mu$ g P/mg of protein		
		Cytoplasm	Mitochondria	Nucleic fraction
Control (intact animals)	8	66.7 $\pm$ 2.8	27.8 $\pm$ 1.7	68.0 $\pm$ 6.8
15 min after injection of epinephrine	8	59.2 $\pm$ 1.6 P = 0.05	21.2 $\pm$ 1.2 P = 0.02	66.4 $\pm$ 7.9
Control	10	64.4 $\pm$ 2.0	34.3 $\pm$ 3.1	77.1 $\pm$ 2.6
After 15 min stimulation	10	53.1 $\pm$ 2.7 P > 0.01	28.0 $\pm$ 3.4 P > 0.2	76.1 $\pm$ 5.8
Control	16	65.8 $\pm$ 1.3	34.0 $\pm$ 3.9	79.8 $\pm$ 2.6
After 3 hr stimulation	16	66.4 $\pm$ 2.0	37.6 $\pm$ 3.6	79.4 $\pm$ 1.8

The quantity of RNA was determined in the subcellular fractions of liver from rats which had received electrical stimulation for 15 min or 3 hr. After stimulation for 15 min the RNA in the cytoplasmic fraction was 17.6 per cent less than the control. During the longer period of stimulation the RNA content of the cytoplasm returned to a normal level (Table 1). The cellular protein content decreased simultaneously with the RNA during the shorter period (15 min) of stimulation and the diminution persisted during the longer period (3 hr) when the RNA content returned to the normal level. After the 3 hr period of stimulation there was a statistically significant decrease in protein content to a level 16.5 per cent lower than in the control animals. The protein

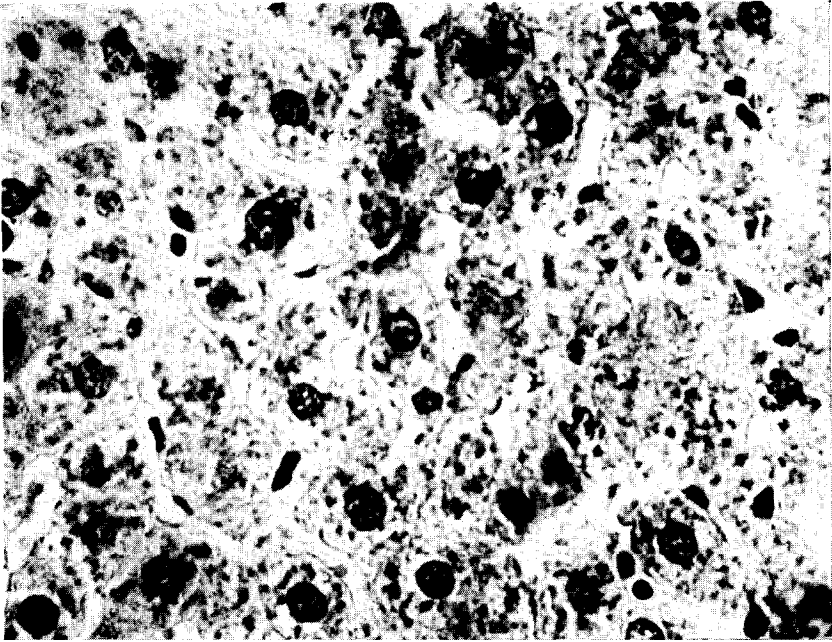


FIG. 1. Liver of rat. Groups of cells with pale tinted and locally imperceptible nuclei. Hematoxylin-eosin stain.  $\times 100$ .

content of the control rat livers was 17.7 (16.4–19.0) mg/100 mg wet wt. and that of the stimulated animals was 14.8 (12.8–16.7) mg/100 mg wet wt., giving  $P = 0.05$ .

No statistically significant changes in the liver mitochondrial or nuclear fractions were observed at either time interval.

### DISCUSSION

The biochemical and histological analyses have shown that treatment of rats with epinephrine leads to the development of dystrophic changes in the hepatic cells and to decrease in the levels of cytoplasmic and mitochondrial RNA. The cytoplasmic RNA was also reduced in the livers of rats given electrical stimulation for 15 min. Comparison of our results with published data on biochemical, histochemical and electron microscopical observations<sup>7, 5</sup> suggests the following hypothesis:

The reduction in RNA content of the cytoplasm of the hepatic cell after a short period of electrical stimulation of the animal may perhaps be related to a labile form of informational RNA. This effect on cytoplasmic RNA may also be related to the observed decrease in total protein of the cell. It may be that the powerful stimulation leads initially to inhibition of synthesis of cytoplasmic RNA which later becomes stabilized by the action of restorative mechanisms. The persistent decrease in total protein after the RNA level has returned to normal suggests changes affecting other fractions of the cellular RNA. The appearance of "smooth membranes" in the endoplasmic reticulum suggests that protein synthesis in the hepatic cell may be inhibited.

The observed biochemical and morphological changes in the liver appear to represent the early reactions of the hepatic cell to strong electrical stimulation of the animal. The similarity in the reactions of the liver cell to injection of epinephrine and to stimulation indicate the important part played by the sympathetic nervous system and catecholamines in these neurogenic dystrophic changes.

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