The Effect of Cyclic N-2-*O*-Dibutyryl-Adenosine-3', 5'-Monophosphate on Neuromuscular Transmission and Concentration of Glycogen in the Isolated Phrenic Nerve-Diaphragm Preparation of the Rat

In a previous work, the interactions of xanthine derivatives, catecholamines and cyclic 3', 5'-adenosine monophosphate (CAMP) has been studied[1]. It was found that CAMP did not affect either the response of the isolated rat diaphragm to indirect or direct stimulation. On the other hand, it has already been suggested that CAMP is able to enhance the release of acetylcholine at the motor nerve endings[2]. This was later supported by the finding that the frequency of miniature end-plate potentials was increased in response to cyclic N–2–*O*-dibutyryl-adenosine-3', 5'-monophosphate (db-CAMP)[3]. Other investigators stated that the quantal release of acetylcholine at the neuromuscular junction is not mediated by CAMP[4].

Taking into account the possible difference in penetration characteristics between CAMP and db-CAMP, it was of interest to compare the effects of these 2 substances on neuromuscular transmission and concentration of glycogen in the isolated phrenic nerve-diaphragm preparation of the rat.

The isolated diaphragm was arranged and stimulated as already described[5]. Contractions of diaphragm were recorded either on a 'Physiograph IV' polygraph, using a photoelectric transducer for quantitative measurements of skeletal muscle contractions (Myograph type A, E and M Company), or on the smoked drum, using an isotonic lever, with a magnification of 8–10 times. Stimulation of the muscle was carried out by square wave pulses delivered from a Grass S-8 stimulator. The frequency of stimulation was 6–10/min, and the duration of pulses was 0.2 to 0.8 msec.

Glycogen in diaphragm was estimated according to the method already described[6]. Pieces of diaphragm, 25–30 mg, were cut out and incubated at 37°C for 10 min in Tyrode solution. Immediately after this period, glycogen was extracted and estimated.

It was found that db-CAMP in concentrations from 1.15×10^{-3} *M* to 3.45×10^{-3} *M* produced potentiation in the response of the isolated diaphragm to indirect stimula-

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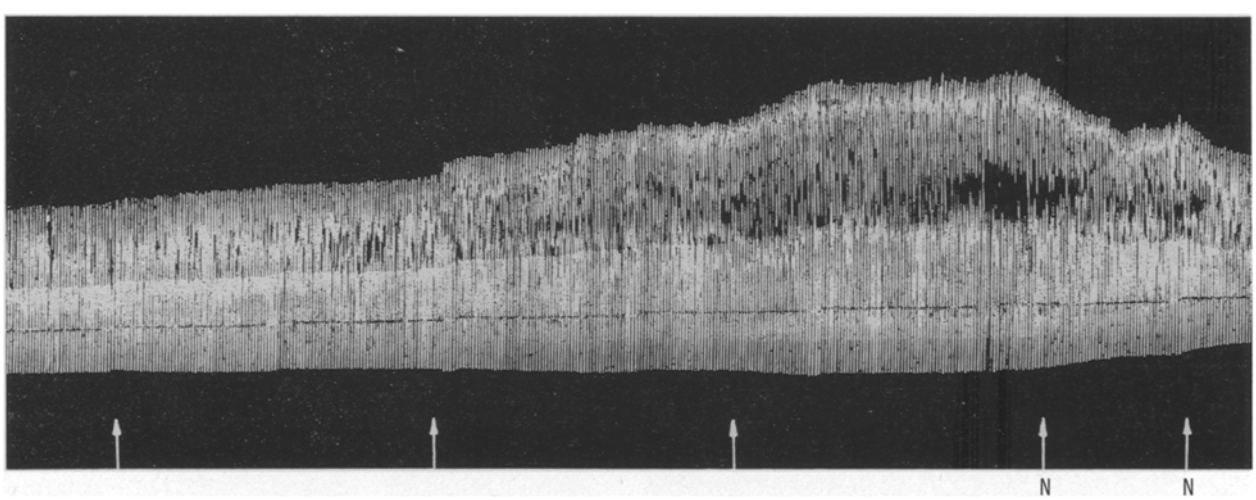

The effect of db-CAMP on the isolated phrenic nerve-diaphragm preparation of the rat. Indirect stimulation at 6/min, 0.2 msec duration. At the arrows, $1.15 \times 10^{-3}M$ db-CAMP added into the bath. At N, 0.25 mg/ml nicotinic acid added into the bath. Speed of the drum: 4 mm/min.

The effect of CAMP, dB-CAMP and adrenaline on the concentration of glycogen in diaphragm of the rat (mg/100 ml)

Control	CAMP ($2 \times 10^{-3}M$)	db-CAMP ($2 \times 10^{-3}M$)	Adrenaline (2×10^{-7} g/ml)	Adrenaline (2×10^{-7} g/ml) + CAMP ($2 \times 10^{-3}M$)	*P*
164 ± 5.2	168 ± 4.5	73 ± 6.4	81 ± 5.1	56 ± 6.4	(1:3) < 0.001 (1:4) < 0.001 (1:5) < 0.001 (2:5) < 0.001 (4:5) < 0.02

The numbers indicate the Mean ± S.E.M. of 5 experiments in each group.

tion in 11 out of 20 experiments. When present, this effect is dose-dependent and it could be antagonized by nicotinic acid (Figure). In contrast to this, CAMP in concentrations from 10^{-5} M to 2×10^{-3} M did not affect the response of the diaphragm to indirect stimulation in all 8 experiments. These findings are in agreement with the action of these 2 nucleotides on the concentration of glycogen in diaphragm (Table). CAMP itself did not change the concentration of glycogen in diaphragm, whereas db-CAMP produced a significant decrease.

It should be pointed out that even CAMP produced potentiation of the diaphragm contractions if added to the bath after previous addition of adrenaline (2×10^{-7} g/ml). Similarly, a potentiating effect of CAMP and adrenaline in producing glycogenolysis in diaphragm was also observed (Table).

These experiments indicate that CAMP might act as a 'second messenger' mediator in producing facilitatory responses of the isolated diaphragm to indirect stimulation. The activated metabolic processes might have the predominant role in this response.

Résumé. Le dérivé dibutyrique du 3′, 5′-AMP cyclique potentialise les contractions du diaphragme provoquées par stimulation électrique indirecte, alors que l'application du 3′, 5′-AMP cyclique n'a pas un tel effet. D'autre part, ce dérivé diminue le taux de glycogène du diaphragme, ce qui n'est pas le cas pour le 3′, 5′-AMP cyclique.

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Albumin Content of Hepatocytes in Experimental Cirrhosis

Serum albumin concentration is often reduced in cirrhosis, and this has been attributed to depression of albumin synthesis following hepatocellular damage[1]. We have employed the fluorescent antibody technique in an attempt to determine whether the production of experimental cirrhosis is accompanied by a reduction in the intracellular albumin content of the liver.

Cirrhosis was induced in 4 male Charles River rats weighing approximately 290 g using the method of MCLEAN et al.[2], sodium phenobarbitone being given for 1 week prior to twice weekly exposure to carbon tetrachloride. This combined treatment was continued for a period of 8 weeks. Since dietary protein intake is known to influence the intracellular albumin content of the liver[3], 4 untreated control rats of the same species, sex and weight were pair fed with the test animals during the entire experimental period of 10 weeks. At the end of the 10th week i.e. 1 week after cessation of treatment both groups of rats were fasted for 2 h and killed under ether anaesthesia. Blocks of liver were immediately taken, fixed in ice-cold 95% ethanol containing 1% acetic acid and processed after the method of HAMASHIMA et al.[4].

Rat albumin was prepared by ammonium sulphate precipitation and then purified by ion-exchange chro-

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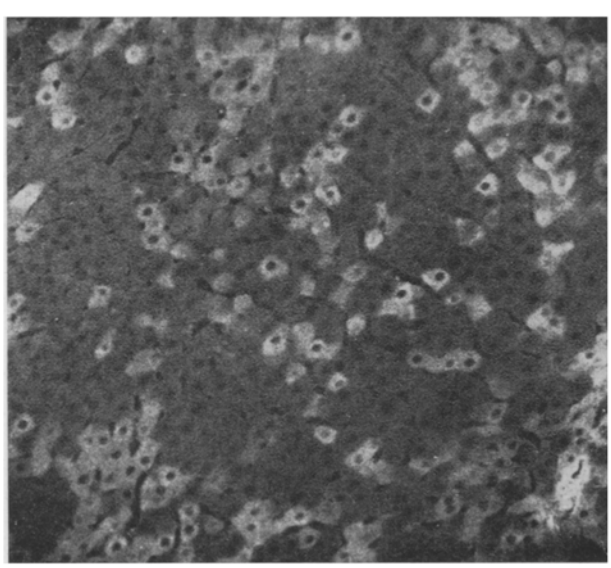

Fig. 1. Normal rat liver showing numerous randomly distributed fluorescent cells. ×163.

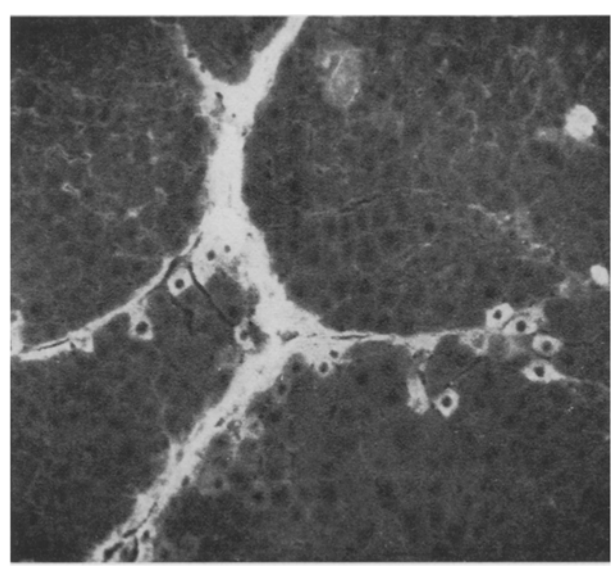

Fig. 2. Cirrhotic rat liver showing only a few fluorescent cells mainly around the periphery of the nodules. Note also the bright staining of the fibrous tissue septa. ×163.