30-min incubation at 37°), uptake of exogenous catecholamine was similarly obtained. This uptake by preincubated subcellular fractions probably reflects the net difference between the rates of release and rates of concentration; interdependent but separate mechanisms may be found to control each of these processes.

The administration of reserpine to the rat (5 mg/kg i.p.) 24 hr prior to the experimental period clearly diminished the uptake-capacity of subcellular fractions prepared from these animals, but did not block it completely (Fig. 2).

These data demonstrate that subcellular fractions of rat brain possess a mechanism for concentrating exogenous noradrenaline similar to that shown for other neural structures.

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REFERENCES

1. A. BERTLER, N. A. HILLARP and E. ROSENGREN, *Acta physiol. scand.* **50**, 113 (1960).
2. H. WEIL-MALHERBE and A. D. BONE, *J. Neurochem.* **4**, 251 (1959).
3. H. BLASCHKO and A. D. WELCH, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **219**, 17 (1953).
4. N. A. HILLARP and B. NILSON, *Acta physiol. scand.* **31**, Supp. 113 (1954).
5. N. A. HILLARP, *Acta physiol. scand.* **47**, 271 (1958).
6. U. S. VON EULER and N. A. HILLARP, *Nature, Lond.* **177**, 44 (1956).
7. H. SCHUMANN, *Naunyn Schmiedebergs Arch. exp. Path. Pharmak.* **234**, 17 (1958).
8. U. S. VON EULER, *Acta physiol. scand.* **43**, 155 (1958).
9. A. BERTLER, A. CARLSSON and E. ROSENGREN, *Acta physiol. scand.* **44**, 273 (1958).
10. U. S. VON EULER and F. LISHAJKO, *Acta physiol. scand.* **51**, 348 (1961).
11. U. S. VON EULER and F. LISHAJKO, *Acta physiol. scand.* **51**, 193 (1961).

The effect of ricinoleic acid and phenolphthalein on creatine phosphate levels in the rat intestine *in vitro*

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MANY recent reports concerning movement of the intestine and intestinal smooth muscle have demonstrated the close relationship between the motility and the energy metabolism.[1-5] Some authors observed[6] that a high concentration of ricinoleic acid and phenolphthalein (10^{-2} to 10^{-3} M) inhibited respiration of intestinal slices of rats, whereas a lower concentration (10^{-6} to 10^{-7} M) stimulated the respiration slightly but significantly. It was probable that the respiratory stimulation by these drugs was caused by an interference with the formation of energy-rich phosphate bonds. The present paper therefore deals with the study of the action of these drugs on creatine phosphate levels in the rat intestine.

After adult albino rats, weighing 150 to 200 g, were sacrificed by decapitation, the intestinal tract was removed, opened lengthwise, and washed with saline. Both small and large intestine were used in this experiment; 300 to 700 mg of slices were incubated in 2 ml of Krebs-Ringer medium at pH 7·4 in which the concentration of Ca^{2+} was 0·001 M. At the end of the incubation period, the pieces of tissue were homogenized with two volumes of 10% trichloroacetic acid at −10° and then centrifuged at 0°. Free and total creatine were determined on aliquots of the supernatant by the colorimetric method of Eggleton *et al.*,[7] as used by Ennor and Rosenberg.[8] The difference between the two was taken as the measure of creatine phosphate. It was confirmed that the color formation was not affected by the concentration of the drugs used.

During 30-min incubation in the medium at 37°, creatine phosphate levels of slices decreased more than 50%. As 0·01 M glucose was added to the medium, the levels did not decrease but slightly increased, although they were lower than those on skeletal, cardiac, and smooth muscle.[4, 9–12]

The experimental results are summarized in Table 1; in the presence of ricinoleic acid and phenolphthalein, creatine phosphate levels in the intestine were always lower than control values. At 10^{-6} M

TABLE 1. EFFECT OF RICINOLEIC ACID, PHENOLPHTHALEIN, AND 2,4-DINITROPHENOL ON CREATINE PHOSPHATE LEVELS OF THE RAT INTESTINE*

		Small intestine		Large intestine	
		Creatine phosphate in tissue (μg/g wet wt)	Total creatine in medium (μg)	Creatine phosphate in tissue (μg/g wet wt)	Total creatine in medium (μg)
Controls	(8)	32·4 ± 6·6	45·4 ± 5·1	65·2 ± 12·7	15·0 ± 2·9
Ricinoleic acid 10^{-6} M	(4)	5·9 ± 5·3	44·6 ± 9·8	23·4 ± 13·1	14·5 ± 0·3
Phenolphthalein 10^{-7} M	(4)	20·5 ± 7·7	60·6 ± 5·8	18·9 ± 13·4	26·3 ± 5·4
2,4-Dinitrophenol 10^{-5} M	(3)	3·2 ± 1·7	56·2 ± 6·0	10·5 ± 5·5	21·6 ± 1·6
2,4-Dinitrophenol 10^{-6} M	(2)	11·2 ± 4·1	49·3 ± 5·2	39·4 ± 13·3	18·3 ± 4·2

* Reaction mixture: 0·01 M glucose, 0·001 M Ca^{2+} in Krebs-Ringer solution; figures within parentheses represent the number of experiments; values are ± standard deviation.

ricinoleic acid the rate of reduction was 82% in the small intestine and 64% in the large; at 10^{-7} M phenolphthalein it was 37% in the small intestine and 71% in the large. 2,4-Dinitrophenol also reduces creatine phosphate levels in the intestine. At the same time it was observed that 2,4-dinitrophenol increased the oxigen consumption by 20 to 30%. Furthermore, with higher concentration of 2,4-dinitrophenol the creatine phosphate levels were not only decreased to a great extent but, also, creatine leaked from the intestine to the medium; 10^{-7} M phenolphthalein, regardless of the rate of the decrease of creatine phosphate, leaked creatine into the medium.

The results of the experiment suggest that the effects of ricinoleic acid and phenolphthalein are the same as those of 2,4-dinitrophenol, which is known to interfere with oxidative phosphorylations, even if leakage of creatine from slices to the medium was also stimulated by phenolphthalein.

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REFERENCES

1. J. R. WEEKS, M. B. CHENOWETH and F. E. SHIDEMAN, *J. Pharmacol. exp. Ther.* **98**, 224 (1950).
2. A. FARAH, T. C. WEST and R. ANGEL, *J. Pharmacol. exp. Ther.* **98**, 234 (1950).
3. G. V. R. BORN and E. BÜLBRING, *J. Physiol., Lond.* **127**, 626 (1955).
4. G. V. R. BORN, *J. Physiol., Lond.* **131**, 704 (1956).
5. E. BÜLBRING and H. LÜLLMAN, *J. Physiol., Lond.* **136**, 310 (1957).
6. T. SUZUKI and S. NAKAO, *Folia pharmacol. jap.* **58**, 106§ (1962).
7. P. EGGLETON, S. R. ELSDEN and N. GOUGH, *Biochem. J.* **37**, 526 (1943).
8. A. H. ENNOR and H. ROSENBERG, *Biochem. J.* **51**, 606 (1952).
9. W. F. H. M. MOMMAERTS, *Phosphorus Metabolism*, vol. 1, p. 552. Johns Hopkins Press, Baltimore (1951).
10. J. M. BARNES, J. I. DUFF and C. J. THRELFALL, *J. Physiol., Lond.* **130**, 585 (1955).
11. J. THOMAS, *Biochem. J.* **64**, 335 (1956).
12. J. E. CREMER, *Biochem. Pharmacol.* **6**, 153 (1961).