Effects of Morphine on Uptake of Glucose and Synthesis of Glycogen in Muscle of Normal and Chronically Morphinized Rats

THERE is as yet no satisfactory explanation for the phenomenon of drug addiction. Morphine-induced biological dependence of a type which might be the basis of addiction in higher animals has not been demonstrated in unicellular organisms which have metabolisms independent of nervous or hormonal control, and no convincing explanation for such induced dependence arises from knowledge of the effects of morphine on the nervous system alone. There is little doubt that the chemical basis of addiction involves hormonal systems, and we further suspect that the site of addicting action may lie in mechanisms whereby hormonal effects are superimposed on the more primitive, intrinsic controlling mechanisms of cellular metabolism.

This view is encouraged by the results of experiments in which, preliminary to a more extensive study of the effects of morphine on the hormonal control of metabolism, we have compared the effects of morphine *in vitro* on the rates of glucose-uptake and glycogen synthesis in excised diaphragms of normal and of chronically morphinized rats.

Experimental procedures were based on those of Gemmill[1,2], Verzár and others[3,4]. Diaphragms from decapitated rats were trisected, the middle, vertebral portion used for the estimation of initial glycogen (we have confirmed the reliability of this procedure) and the two similar lateral portions were incubated in separate vessels for 2 h at 37° and *p*H 7·4 in aerated, phosphate-buffered saline (2 ml.) containing glucose (0·15 per cent), one containing morphine. Chronically morphinized rats were rats which had received daily injections of morphine (0·3 mg/100 g body-wt.) for 6 weeks. The control rats had received daily injections of saline for the same period. Glycogen was determined by the method of Good *et al.*[5] and glucose by Nelson's method[6].

The rate of uptake of glucose by diaphragms of normal rats was increased by the presence of morphine ($1{\cdot}9 \times 10^{-4} - 1{\cdot}6 \times 10^{-2}$ M) in the buffered glucose–saline medium. In some experiments a four-fold increase was observed and in no case was the rate of glucose-uptake decreased by the addition of morphine. On the other hand, addition of morphine to diaphragms of chronically morphinized rats decreased the rate of glucose-uptake. The effects of morphine in a bicarbonate-buffered medium were similar to those in the phosphate-buffered medium. The results of experiments with $7{\cdot}7 \times 10^{-4}$ M final concentration of morphine are expressed in Table 1.

In the absence of added morphine there is no significant difference between the rates of glucose-uptake by diaphragms of normal and of chronically morphinized rats. This is confirmed by comparing chronically morphinized rats with control rats which had received saline injections (Table 2). On the other hand, the mean glycogen content of the diaphragms of the morphinized rats is somewhat higher than is that of the saline-injected controls ($P < 0{\cdot}1 > 0{\cdot}05$).

Table 1. EFFECTS OF MORPHINE ($7{\cdot}7 \times 10^{-4}$ M) ON GLUCOSE-UPTAKE BY DIAPHRAGMS OF NORMAL AND CHRONICALLY MORPHINIZED RATS

	Glucose-uptake, mg/g wet tissue/h Mean ± *S.E.* (range)			
	Control	+Morphine	Difference	*t*-test
Normal rats (21)	1·58 ± 0·141 (0·34–3·00)	2·58 ± 0·204 (1·45–4·00)	+1·0 ± 0·192	($P < 0{\cdot}001$)
Chronically morphinized rats (6)	2·46 ± 0·452 (0·98–4·08)	1·73 ± 0·355 (0·66–3·14)	−0·73 ± 0·269	($P < 0{\cdot}05$)

Table 2. INITIAL GLYCOGEN CONTENT AND RATE OF GLUCOSE-UPTAKE OF DIAPHRAGMS OF NORMAL AND CHRONICALLY MORPHINIZED RATS

	Glycogen, mg/g wet tissue Mean ± *S.E.* (range)	Glucose-uptake mg/g wet tissue/h Mean ± *S.E.* (range)
Control rats, saline-injected (12)	1·03 ± 0·145 (0·71–2·16)	1·83 ± 0·239 (0·96–3·54)
Chronically morphinized rats (16)	1·43 ± 0·157 (0·70–3·14)	1·87 ± 0·249 (0·38–4·08)

The rates of change of glycogen content during incubation of diaphragms of both normal and chronically morphinized rats are less affected by added morphine than are the rates of glucose-uptake. The mean rate of glycogen 'synthesis' in 9 normal rats was increased by addition of morphine ($7{\cdot}7 \times 10^{-4}$ M) from 0·275 ± 0·132 to 0·347 ± 0·127 mg/g tissue/h, but we cannot claim that the difference, +0·072 ± 0·162, is significant. In 6 chronically morphinized rats the mean rate of glycogen synthesis was decreased by addition of morphine from 0·798 ± 0·206 to 0·398 ± 0·246, the mean difference being −0·40 ± 0·189 ($P < 0{\cdot}1 > 0{\cdot}05$). It would appear that the effects of morphine on glycogenesis or on glycogenolysis are secondary to direct effects on glucose-uptake. This view is further supported by our observations that morphine does not influence the rates of oxygen consumption and that it has relatively negligible effect on lactate production *in vitro* by diaphragms of both normal and chronically morphinized rats.

We thank the China Medical Board of New York, Inc., for grants for the purchase of apparatus.

C. H. LEE PENG
E. O'F. WALSH

Department of Biochemistry,
University of Hong Kong.

[1] Gemmill, C. L., *Bull. Johns Hopk. Hosp.*, **66**, 232 (1940).
[2] Gemmill, C. L., *Bull. Johns Hopk. Hosp.*, **68**, 329 (1940).
[3] Verzár, F., and Wenner, V., *Biochem. J.*, **42**, 35 (1948).
[4] Leupin, E., and Verzár, F., *Biochem. J.*, **46**, 562 (1950).
[5] Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, **100**, 485 (1933).
[6] Nelson, N., *J. Biol. Chem.*, **153**, 375 (1944).

Molecular Weights of Enzymes

IN a recent article Wright[1] put forward the hypothesis that the molecular weights of the enzymes tabulated by Dixon and Webb[2] fall near three series of numbers expressed by $2^n \times 12{,}000$, $2^n \times 16{,}000$ and $2^n \times 19{,}000$, n taking integral values 0–4. This hypothesis was based entirely on the experimental values themselves, mainly by means of graphical representation. A numerical test of the degree of fit of the data to the hypothesis was applied and indicated a good agreement; however, since this involved the arrangement of experimental values into groups, it contained the possibility of prejudice. It therefore seemed worth while to apply the more exacting method of the 'correlation function' used by Johnston, Longuet-Higgins and Ogston[3] to test the Svedberg hypothesis[4].

For this purpose, the null hypothesis is that values of log M are rectangularly distributed within the interval between the two successive predicted log values in which each falls. The predicted log values were those of Wright's hypothesis; the 59 experimental values used were taken from Dixon and Webb's