Biochemical Pharmacology, 1963, Vol. 12, pp. 921–930. Pergamon Press Ltd., Printed in Great Britain.

EFFECTS OF MORPHINE ON THE HORMONAL CONTROL OF METABOLISM

I. *IN VITRO* EFFECTS OF MORPHINE AND HYDROCORTISONE ON UTILIZATION OF GLUCOSE BY MUSCLE OF NORMAL AND CHRONICALLY MORPHINISED RATS

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

Department of Biochemistry, University of Hong Kong, Hong Kong

(*Received* 9 *April* 1963; *accepted* 23 *May* 1963)

Abstract—The effects of morphine and of hydrocortisone, acting singly and together, on glucose-uptake, glycogen metabolism, glycolysis, and respiration of excised diaphragms of normal and of chronically morphinised rats have been studied *in vitro*.

It is demonstrated that morphine stimulates glucose-uptake of normal tissue but depresses glucose-uptake and lactate production in chronically morphinised tissue, that hydrocortisone antagonises these effects of morphine, and that morphine antagonises the effect of hydrocortsone on respiration but tends to enhance the effects of the hormone on glycogen metabolism.

Diaphragm from chronically morphinised animals has a higher glycogen content but is no different from that of normal animals in basal metabolic activity. It is concluded that morphine-induced changes resulting in differences in sensitivity and response to both hormone and drug involve mechanisms whereby hormonal effects are superimposed on the intrinsic controlling systems of cellular metabolism.

THE morphine molecule embodies an oxygenated, partially reduced phenanthrene ring system and in this respect it shows a structural resemblance to the steroids. It also embodies an adrenaline-like structure and, like adrenaline, it is a biological derivative of tyrosine.[1, 2] We suspect that it is this embodiment in one molecule of structures and functional groups analogous to two types of hormone, which are believed to act synergistically,[3] that endows morphine with its peculiar addicting properties and, as previously suggested,[4] that a site of addicting action may lie in mechanisms whereby these hormones exert their influence on cellular metabolism.

It is known that administration of morphine affects adrenal function *in vivo*, influencing secretion of both corticosteroids[5] and catecholamines.[6] It is also known that adrenalectomy strongly influences the sensitivity of an animal's response to morphine[7, 8] and that administration of corticosteroids or of adrenocorticotrophic hormone modifies response to analgesic drugs.[9, 10] In general, these effects *in vivo* are attributed to the drug's acting either directly on the endocrines or through the nervous system.

We here report the results of a study of the effects *in vitro* of morphine and of hydrocortisone, singly and together, on the rates of glucose-uptake, glycogen metabolism, lactate production, and oxygen-consumption by isolated diaphragm of both normal and chronically morphinised rats. The results of these experiments demonstrate that

morphine has a direct and hormone-simulating effect on tissue metabolism which is opposed by hydrocortisone, that morphine can antagonise and also induce a sensitivity to hydrocortisone, and that chronic morphinisation induces changes whereby tissue response either to the hormone or the drug is different from that of normal tissue.

MATERIALS AND METHODS

Pretreatment of animals

Female albino rats (140–180 g) were used. They were maintained on a standard diet *ad libitum* but were fasted 24 hr before decapitation prior to excision of the diaphragm.

Chronically morphinised rats had received daily injections of 1% morphine sulphate (0·4–0·6 ml ≡ 30 mg drug/kg body wt) for 5–6 weeks. Control rats had received daily injections of saline for the same period.

Experimental procedure

Freshly excised diaphragms were rinsed in ice-cold incubation medium, soaked (15 min) in fresh ice-cold medium (30 ml), bisected, after removal of the middle vertebral portion when initial glycogen determinations were required.[11] The two similar lateral portions having been blotted "dry" and weighed were incubated at pH 7·4 and 37° in separate vessels in oxygenated phosphate-buffered saline (2·0 ml) containing glucose (0·15%) ± drug and/or hormone (either, or each, in final concentration $7{\cdot}7 \times 10^{-4}$ M).

Glucose uptake and lactate production were determined by changes in concentration in the medium during the experimental period (2 hr). Changes in glycogen content of the tissue were estimated by the differences between the initial glycogen content of the middle vertebral portion of the diaphragm and the final glycogen contents of the lateral portions at the end of the experiment. Oxygen uptakes during 1 hr were measured by the direct method in the Warburg apparatus with oxygen as gas phase, the centre wells containing 30% KOH (0·2 ml).

Incubation media

Krebs–Ringer–phosphate saline as modified by Herman and Ramey,[12] containing glucose (0·15%), pH 7·4, was used with one or more of the following additives: 0·0154 M morphine, prepared by diluting 0·154 M morphine sulphate with 0·154 M NaCl; 0·0154 M hydrocortisone in 50% ethanol, and an equal amount of 0·154 M NaCl and/or 50% ethanol in the controls.

pH values were checked using a Pye Dynacap pH-meter.

Analytical methods

Glucose was determined by the method of Nelson[13] after deproteinisation of the sample (0·20 ml) with $Ba(OH)_2$ and $ZnSO_4$.

Glycogen was determined by conversion to glucose by the method of Good *et al.*[14] and the glucose determined by the Nelson[13] method. For this estimation the whole, weighed portion of diaphragm was digested in 30% KOH (0·5 ml).

Lactate was determined by the method of Barker and Summerson[15] after deproteinisation of the sample (0·50 ml) with 5% trichloroacetic acid (0·50 ml).

A Hilger "Uvispek" spectrophotometer and a Spekker absorptiometer were used for spectrophotometric and colorimetric determinations.

RESULTS

Effects of morphine and hydrocortisone on glucose-uptake by isolated diaphragm of normal and chronically morphinised rats

The mean rate of glucose-uptake *in vitro* by diaphragm of chronically morphinised rats is not significantly different from that of control rats which had received saline injections daily during the morphinisation period. Thus for thirty-three control rats the mean rate ±S.E. mg glucose/100 g wet tissue per hr was 223 ± 20 and for 28 chronically morphinised rats it was 180 ± 19 (difference, 43 ± 28). On the other hand, the mean rate of glucose-uptake *in vitro* in the presence of morphine ($7{\cdot}7 \times 10^{-4}$ M) is considerably lower in the diaphragm of chronically morphinised rats than in that of control rats. Thus, for thirty-two control rats it was 259 ± 16 and for 15 chronically morphinised rats it was 134 ± 23 (difference, 125 ± 29, $P > 0{\cdot}001$).

TABLE 1. EFFECTS OF MORPHINE AND HYDROCORTISONE ON UPTAKE OF GLUCOSE BY ISOLATED DIAPHRAGM OF NORMAL AND OF CHRONICALLY MORPHINISED RATS

Hemi-diaphragms were incubated at pH 7·4 and 37° for 2 hr in oxygenated Krebs–Ringer–phosphate (2·0 ml) containing glucose (0·15%) ± added hormone or drug (final concentration, $7{\cdot}7 \times 10^{-4}$ M). In each experiment, half the excised diaphragm served as the control for the other half. Mean rates ± S.E. mean are expressed as decrease in glucose content of the medium, mg/100 g wet tissue per hr.

State of rat	Number of rats	Control	Experiment	Difference
			+ Morphine	
Normal	(12)	179 ± 18	288 ± 21	+109 ± 25 ($P = 0{\cdot}001$)
Chronically morphinised	(7)	231 ± 41	159 ± 30	−72 ± 22 ($P < 0{\cdot}02$)
			+ Hydrocortisone	
Normal	(11)	264 ± 34	228 ± 41	−36 ± 32
Chronically morphinised	(12)	139 ± 28	134 ± 46	−5 ± 44
			+ Morphine + Hydrocortisone	
Normal	(10)	230 ± 48	369 ± 60	+139 ± 33 ($P < 0{\cdot}002$)
Chronically morphinised	(9)	194 ± 27	168 ± 32	−26 ± 46
		+ Hydrocortisone	+ Morphine + Hydrocortisone	
Normal	(7)	222 ± 51	147 ± 59	−75 ± 89
Chronically morphinised	(9)	92 ± 42	138 ± 54	+46 ± 62
		+ Morphine	+ Morphine + Hydrocortisone	
Normal	(11)	260 ± 30	150 ± 28	−110 ± 48 ($P < 0{\cdot}05$)
Chronically morphinised	(8)	112 ± 34	186 ± 27	+74 ± 34 ($P \simeq 0{\cdot}05$)

As shown in the "paired technique" experiments (Table 1), morphine increases the rate of glucose-uptake by normal rat-diaphragm and it decreases the rate in the chronically morphinised rat-diaphragm. That this latter effect is not caused simply by the presence of a higher concentration of morphine is indicated by the fact, as reported in a preliminary communication,[4] that much higher concentrations of morphine (up to $1{\cdot}6 \times 10^{-2}$ M) also stimulate glucose-uptake in normal diaphragm.

Addition of hydrocortisone ($7{\cdot}7 \times 10^{-4}$ M) to the medium has no significant effect on rates of glucose-uptake by diaphragms of either normal or chronically morphinised animals under our conditions of experiment, but it opposes the effects of added morphine. As illustrated in Table 1, hydrocortisone tends to depress glucose-uptake of morphine-stimulated normal diaphragm and to stimulate glucose-uptake of morphine-depressed, chronically morphinised diaphragm.

Effect of morphine and hydrocortisone on glycogen metabolism in isolated diaphragm of normal and chronically morphinised rats

The mean glycogen content of freshly excised diaphragms of fasted, chronically morphinised rats is appreciably higher than that of fasted, normal rats. Thus, for

TABLE 2. EFFECTS OF MORPHINE AND HYDROCORTISONE ON GLYCOGEN METABOLISM IN ISOLATED DIAPHRAGM OF NORMAL AND OF CHRONICALLY MORPHINISED RATS

In each experiment, the freshly excised diaphragm was trisected, the middle, vertebral portion used for estimation of initial glycogen content and the two similar, lateral portions, one serving as control for the other, were incubated separately at pH 7·4 and 37° for 2 hr in oxygenated, Krebs–Ringer–phosphate (2·0 ml) containing glucose (0·15%) ± added hormone or drug (final concentration, $7{\cdot}7 \times 10^{-4}$ M). Mean rates of change of glycogen content ± S.E. mean are expressed as increase (+) or decrease(−) of glucose equivalent, mg/100 g wet tissue per hr.

State of rat	Number of rats	Control	Experiment	Difference
			+ Morphine	
Normal	(9)	+28 ± 13	+35 ± 13	+7 ± 16
Chronically morphinised	(8)	+62 ± 19	+32 ± 19	−30 ± 16 ($P < 0{\cdot}1$)
			+ Hydrocortisone	
Normal	(8)	−13 ± 10	−26 ± 10	−13 ± 4 ($P < 0{\cdot}01$)
Chronically morphinised	(10)	+27 ± 4	+7 ± 6	−21 ± 5 ($P \simeq 0{\cdot}002$)
			+ Morphine + Hydrocortisone	
Normal	(11)	+43 ± 8	+11 ± 12	−32 ± 5 ($P \ll 0{\cdot}001$)
Chronically morphinised	(9)	−6 ± 9	−31 ± 12	−25 ± 11 ($P < 0{\cdot}05$)
		+ Hydrocortisone	+ Morphine + Hydrocortisone	
Normal	(6)	−8 ± 7	−6 ± 7	+1 ± 1
Chronically morphinised	(9)	−33 ± 8	−36 ± 9	−3 ± 4
		+ Morphine	+ Morphine + Hydrocortisone	
Normal	(11)	+40 ± 5	+5 ± 4	−35 ± 5 ($P \ll 0{\cdot}001$)
Chronically morphinised	(10)	+3 ± 8	−19 ± 5	−22 ± 7 ($P < 0{\cdot}01$)

thirty-four control rats the mean initial glycogen content ± S.E., mg glycogen/100 g wet tissue was 65 ± 9 and for 32 chronically morphinised rats it was 118 ± 14 (difference, 53 ± 16, $P = 0{\cdot}002$).

In the experiments here reported, the mean rate of increase of glycogen content during 2 hr incubation *in vitro* at 37° and pH 7·4 in oxygenated, phosphate-buffered

saline containing glucose was also, though not significantly, higher in the chronically morphinised than in the control rat diaphragms. Thus, for twenty-eight control rats the mean rate of increase ± S.E., mg glycogen/100 g wet tissue per hr was 22 ± 7 and for twenty-seven chronically morphinised rats it was 27 ± 8 (difference, 5 ± 11).

As shown in the "paired technique" experiments (Table 2), morphine tends slightly to stimulate the rate of increase of glycogen in diaphragms of normal rats and to depress the rate in those of chronically morphinised rats, but these effects are small and are presumably secondary to the effects of morphine on glucose-uptake.

Hydrocortisone markedly decreases the rate of glycogenesis in diaphragms of both normal and chronically morphinised rats and from the results listed in Table 2 there is a suggestion that in the normal rat-diaphragm this effect of hydrocortisone is enhanced by the presence of morphine.

Effects of morphine and hydrocortisone on lactate accumulation in isolated diaphragm of normal and chronically morphinised rats

The rate of lactate accumulation *in vitro* by diaphragm of chronically morphinised rats is not significantly different from that of diaphragm of normal rats and rates are not significantly affected by hydrocortisone. Thus, for twenty-eight control rats the mean rate ± S.E. of increases of lactate during 2 hr incubation *in vitro* at 37° and

TABLE 3. EFFECTS OF MORPHINE AND HYDROCORTISONE ON LACTATE ACCUMULATION IN ISOLATED DIAPHRAGM OF NORMAL AND OF CHRONICALLY MORPHINISED RATS

Hemi-diaphragms were incubated at pH 7·4 and 37° for 2 hr in oxygenated Krebs–Ringer–phosphate (2·0 ml) containing glucose (0·15%) ± added hormone or drug (final concentration, $7{\cdot}7 \times 10^{-4}$ M). In each experiment, half the excised diaphragm served as the control for the other half. Mean rates ± S.E. mean are expressed as increase in lactic acid content of the medium, mg/100 g wet tissue per hr.

State of rat	Number of rats	Control	Experiment	Difference
			+ Morphine	
Normal	(10)	83 ± 12	82 ± 11	−1 ± 13
Chronically morphinised	(12)	102 ± 12	75 ± 8	−27 ± 12 ($P < 0{\cdot}05$)
			+ Hydrocortisone	
Normal	(11)	92 ± 5	97 ± 5	+5 ± 6
Chronically morphinised	(8)	84 ± 14	87 ± 10	+3 ± 14
			+ Morphine + Hydrocortisone	
Normal	(7)	118 ± 14	126 ± 11	+8 ± 9
Chronically morphinised	(12)	119 ± 11	124 ± 12	+5 ± 8
		+ Hydrocortisone	+ Morphine + Hydrocortisone	
Normal	(6)	97 ± 10	103 ± 9	+6 ± 6
Chronically morphinised	(9)	120 ± 9	123 ± 22	+3 ± 18
		+ Morphine	+ Morphine + Hydrocortisone	
Normal	(11)	128 ± 13	125 ± 9	−3 ± 15
Chronically morphinised	(11)	74 ± 10	94 ± 14	+20 ± 11 ($P < 0{\cdot}1$)

of 7·4 in oxygenated, phosphate-buffered saline containing glucose was 95 ± 6 mg lactic acid/100 g wet tissue per hr and for thirty-two chronically morphinised rats it was 104 ± 7 (difference, 9 ± 9). In the presence of hydrocortisone ($7·7 \times 10^{-4}$ M), values were for seventeen control rats, 97 ± 5 and for 17 chronically morphinised rats, 105 ± 8 (difference, 8 ± 9).

In the presence of morphine, however, the rate of lactate accumulation *in vitro* by diaphragm of chronically morphinised rats is lower than that by diaphragm of normal rats. In the presence of morphine ($7·7 \times 10^{-4}$ M) values were 105 ± 10 for twenty-two normal rats and 74 ± 6 for twenty-three chronically morphinised rats (difference, 31 ± 12, $P < 0·02$).

As shown in the "paired technique" experiments (Table 3), addition of morphine *in vitro* does not influence the rate of lactate accumulation by normal diaphragm but it tends to depress the rate by chronically morphinised diaphragm. Hydrocortisone opposes this effect of morphine.

TABLE 4. EFFECTS OF MORPHINE AND HYDROCORTISONE ON UPTAKE OF OXYGEN BY ISOLATED DIAPHRAGM OF NORMAL AND OF CHRONICALLY MORPHINISED RATS

Hemi-diaphragms were incubated with shaking in oxygenated Krebs–Ringer–phosphate (2·0 ml) containing glucose (0·15%) ± added hormone or drug (final concentration, $7·7 \times 10^{-4}$ M) at pH 7·4 and 37° for 1 hr. O_2-uptakes were measured by the Warburg direct method with O_2 as gas phase, the centre wells containing 30% KOH (0·2 ml). In each experiment half the excised diaphragm served as control for the other half. Mean rates ± S.E. mean are expressed as μl O_2/g wet tissue per hr.

State of rat	Number of rats	Control	Experiment	Difference
			+ Morphine	
Normal	(9)	1,375 ± 71	1,401 ± 71	+27 ± 75
Chronically morphinised	(6)	1,550 ± 88	1,589 ± 48	+24 ± 39
			+ Hydrocortisone	
Normal	(8)	1,162 ± 42	1,026 ± 48	−136 ± 35 ($P < 0·01$)
Chronically morphinised	(6)	1,405 ± 108	1,007 ± 94	−398 ± 75 ($P < 0·01$)
			+ Morphine + Hydrocortisone	
Normal	(5)	1,528 ± 200	1,376 ± 159	−152 ± 125
Chronically morphinised	(10)	1,265 ± 49	1,058 ± 58	−207 ± 70 ($P < 0·02$)
		+ Hydrocortisone	+ Morphine + Hydrocortisone	
Normal	(8)	992 ± 115	1,085 ± 110	−163 ± 79
Chronically morphinised	(8)	1,096 ± 95	1,248 ± 124	−152 ± 101
		+ Morphine	+ Morphine + Hydrocortisone	
Normal	(12)	1,176 ± 63	980 ± 69	−196 ± 36 ($P < 0·001$)
Chronically morphinised	(8)	1,220 ± 92	1,038 ± 79	−182 ± 107

Effects of morphine and hydrocortisone on oxygen-uptake by isolated diaphragm of normal and of chronically morphinised rats

The rate of oxygen-uptake *in vitro* by diaphragms of chronically morphinised rats is not significantly different from that of diaphragms of normal rats and the rates are

not significantly affected by morphine. Thus, for twenty-two control rats the mean rate ± S.E. of oxygen-uptake during 1 hr at 37° and pH 7·4 in oxygenated, phosphate-buffered saline containing glucose was 1332 ± 61 μl O_2/g wet tissue per hr and for twenty-two chronically morphinised rats it was 1381 ± 49 (difference, 49 ± 78). In the presence of morphine ($7{\cdot}7 \times 10^{-4}$ M), values were for twenty-one control rats, 1273 ± 52 and for fourteen chronically morphinised rats, 1372 ± 83 (difference 99 ± 92). In the presence of hydrocortisone rates of oxygen-uptake were somewhat lower but no difference between normal and chronically morphinised rats is observed. Thus, in presence of hydrocortisone ($7{\cdot}7 \times 10^{-4}$ M), values were for sixteen control rats 974 ± 61 and for fourteen chronically morphinised rats, 1058 ± 71 (difference, 84 ± 94).

As shown in the "paired technique" experiments (Table 4), addition of morphine *in vitro* does not influence the rates of oxygen-uptake by either normal or chronically morphinised diaphragm. Addition of hydrocortisone *in vitro* significantly depresses the rates of oxygen-uptake and it would seem that the chronically morphinised diaphragm is somethwat more sensitive to this effect of hydrocortisone than is the normal diaphragm. Morphine opposes the effects of hydrocortisone on oxygen-uptake to a certain extent, but in the equimolecular concentrations of drug and hormone here used it does not abolish the hormonal effect completely.

DISCUSSION

That morphine has a hormone-simulating effect in accelerating the uptake of glucose by muscle and that chronic morphinisation *in vivo* induces a change whereby the acute effect of the drug on the tissue *in vitro* is different from that of normal tissue is clearly demonstrated.

These findings encourage the view that drug-additiction is associated with adaptive changes in cells and that in the case of morphine hormonal mechanisms are involved.[4] It would be premature at this stage of the investigation, however, to attempt an explanation of the mode of action of morphine in its effects on uptake of glucose. The mechanism whereby glucose-uptake of muscle cells is controlled and influenced by hormones has yet to be elucidated, and it is by no means certain whether a variably selective membrane-permeability or a variably active enzymic system is the major controlling factor.

Morphine has no appreciable effect on the enzymic systems of glycolysis or cellular respiration, as the present work confirms, and cumulative evidence including earlier studies with habit-forming and addicting drugs[16–22] has indicated that effects on intact cells are often greater than can be accounted for by the effects on isolated enzymes. Not all enzymic systems have been individually tested, however, and possibly some which might be highly sensitive to morphine have yet to be discovered, but there is good reason to suspect that the effects of morphine on glucose-uptake are associated with activity at the membrane level.

That morphine, in its influence on uptake of glucose, should have the opposite effect on tissue from a chronically morphinised animal is intriguing. It implies that some change, possibly in the membrane itself, has been induced, but until we have a better understanding of the actual mechanism involved and of the effects of morphine in relation to and in combination with all the hormones which influence the system, there

is little to be gained by speculating on the nature of such change. As a contribution towards the knowledge required we have compared the effects of morphine with those of a glucocorticoid in similar concentration on glucose-uptake, change of glycogen content, lactate accumulation, and oxygen-consumption of both normal and chronically morphinised rat-diaphragms. We have extended the comparison to include the effects of the drug and hormone together, of the effect of the drug in the presence of added hormone, and of the hormone in the presence of added drug.

The present work shows that the dominant effects of the drug and of the hormone on carbohydrate metabolism are quite different, morphine strongly influencing glucose-uptake and hydrocortisone strongly influencing glycogen metabolism. Although, under the conditions of our experiments, hydrocortisone alone has no influence on the rates of glucose-uptake, it antagonises the effects of morphine in diaphragm of both normal and chronically morphinised rats. On the other hand, morphine does not oppose the effect of hydrocortisone on glycogen metabolism. The balance between glycogenesis and glycogenolysis, as measured by the rate of change in glycogen content, is shifted by hydrocortisone in favour of glycogenolysis, possibly by retarding glycogenesis.[11, 23] Morphine would appear to enhance rather than to oppose this effect of the hormone. There is no evidence that morphine has any direct effect on glycogen metabolism since such effects as are observed in some experiments, especially in the chronically morphinised diaphragm, are probably secondary to the effects on glucose-uptake. In contrast, the effects of hydrocortisone on glycogen metabolism appear to be direct and independent of glucose-uptake.

In the effects on lactate production we again find a hypersensitivity of the tissue from the chronically morphinised animal, not to the hormone as in the case of glycogen metbolism, but to the drug. Morphine depresses the rate of lactate accumulation in the chronically morphinised diaphragm and hydrocortisone, which alone has no significant effect, opposes this action of the drug.

Antagonism between the drug and hormone is again observed in the effects on respiration of the tissue. Here, hydrocortisone depresses respiration and the tissue from chronically morphinised animals appears to be hypersensitive to this action of the hormone. Morphine alone is without significant effect on the rate of oxygen consumption by diaphragm of either normal or chronically morphinised animals, but it tends to oppose the action of hydrocortisone. Hydrocortisone inhibits respiration of liver mitochondria, according to Gallangher[24] by increasing membrane permeability thus allowing loss by diffusion of soluble respiratory cofactors. Presumably, the site of action of hydrocortisone on muscle respiration is also the mitochondrial membrane. There is no evidence, however, that morphine acts directly on this membrane, for it has no direct influence on cellular respiration, but it apparently interferes with the action of hydrocortisone at the mitochondrial membrane, just as the hormone appears to block the action of the drug at the cell membrane.

On the whole, the evidence available at present suggests that morphine exerts its major influence at the cell membrane where it influences glucose-uptake, while hydrocortisone exerts its major influence not on the cell membrane but on the mitochondrial membrane and other systems within the cell. Though the drug and hormone act on different systems they each interfere with the action of the other at the membrane level. In their effects on glycogen metabolism and glycolysis there is no evidence that

membranes are directly involved and the observations that morphine does not antagonise the effect of hydrocortisone on glycogenesis is not inconsistent with the above interpretation.

There is no simple explanation in terms of basic metabolic processes for the observed differences between tissues of normal and of chronically morphinised animals. The mean basic rates of glucose-uptake, of net glycogen synthesis, of lactate accumulation, and of respiration are essentially the same in both normal and chronically morphinised diaphragms. These observations alone suggest that any changes induced by repeated dosage with morphine do not involve, as far as carbohydrate metabolism is concerned, serious enzymic changes or modifications in the intrinsic controlling mechanisms of cellular metabolism. Explanation for such differences as are observed must be sought in mechanisms whereby the effects of external factors, such as hormones or drugs, are superimposed on the intrinsic control of metabolism in the cell.

The most striking difference between normal and chronically morphinised tissue is observed in the response to morphine, and to hydrocortisone in the presence of morphine, of the mechanism controlling rate of glucose-uptake, a site for the interplay of various hormonal influences. Another marked difference is in glycogen content, which is significantly higher in diaphragms from chronically morphinised animals. Here, too, the balance between glycogen synthesis and breakdown is subject to hormonal control and, in this case, hydrocortisone is one of the hormones directly involved. Since morphine has no marked direct effect on glycogen metabolism and the effect of morphine on the chronically morphinised diaphragm is to depress glucose-uptake (we have as yet no knowledge of possible effects on glyconeogenesis), it seems likely that the higher glycogen content of the chronically morphinised tissue is a result of a change in the balance of hormones which influence glycogenesis and glycogenolysis. Possibly, a morphine-induced change in response to hormones other than hydrocortisone is involved, but it is well known that adrenal function is disturbed by repeated dosage with morphine.[5, 6]

Yet another difference is the hypersensitivity of the chronically morphinised tissue to the influence of hydrocortisone, a condition possibly analogous to the increased sensitivity to steroids of diaphragms from adrenally insufficient animals, which Huisman[25] observed and which Ramey and Goldstein[3] point out is consistent with the common observation that hormonal deficiency produces increased sensitivity to that hormone.

The present work shows that morphine acts not only on the nervous system and encrocrines but also on target sites of hormonal action and that changes are induced in such sites by chronic administration of the drug. Such changes may well contribute to the establishment of addiction.

Analogous studies with morphine and adrenaline and with adrenaline and hydrocortisone are in progress.

Acknowledgements—The authors gratefully acknowledge gifts of equipment from the China Medical Board of New York, Inc.

REFERENCES

1. A. R. Battersby and B. J. T. Harper, *Chem. & Ind.* **364** (1958).
2. A. R. Battersby and R. Binks, *Proc. Chem. Soc.* **360** (1960).
3. E. R. Ramey and M. S. Goldstein, *Physiol. Rev.* **37**, 155 (1957).
4. C. H. Lee Peng and E. O'F. Walsh, *Nature, Lond.* **196**, 171 (1962).

5. A. J. Eisenmann, H. Isbell, H. F. Frazer and J. Sloan, *Fed. Proc.* **12**, 200 (1953).
6. L. M. Gunne, *Nature, Lond.* **184**, 1950 (1959); **195**, 815 (1962).
7. T. K. Adler, H. W. Elliott and R. George, *J. Pharmacol. exp. Ther.* **120**, 475 (1957).
8. E. L. Way, Y. S. Chen and J. M. Fujimoto, *J. Pharmacol. exp. Ther.* **110**, 51 (1954).
9. C. A. Winter and L. Flataker, *J. Pharmacol. exp. Ther.* **103**, 93 (1951).
10. H. W. Elliott and C. Elison, *Fed. Proc.* **18**, 387 (1959).
11. E. Leupin and F. Verzár, *Biochem. J.* **46**, 562 (1950).
12. M. S. Herman and E. R. Ramey, *Amer. J. Physiol.* **199**, 226 (1960).
13. N. Nelson, *J. biol. Chem.* **153**, 375 (1944).
14. C. A. Good, H. Kramer and M. Somogyi, *J. biol. Chem.* **100**, 485 (1933).
15. S. B. Barker and W. H. Summerson, *J. biol. Chem.* **138**, 535 (1941).
16. B. E. Ryman and E. O'F. Walsh, *Nature, Lond.* **167**, 770 (1951).
17. B. E. Ryman and E. O'F. Walsh, *Biochem. J.* **50**, 570 (1952).
18. B. E. Ryman and E. O'F. Walsh, *Biochem. J.* **58**, 111 (1954).
19. A. R. Fahmy and E. O'F. Walsh, *Biochem. J.* **58**, 231 (1954).
20. A. R. Fahmy, B. E. Ryman and E. O'F. Walsh, *J. Pharm. Pharmacol.* **6**, 607 (1954).
21. A. R. Fahmy and E. O'F. Walsh, *Nature, Lond.* **173**, 872 (1954).
22. S. G. Kalyanpur and E. O'F. Walsh, *Nature, Lond.* **188**, 939 (1960).
23. F. Verzár and V. Wenner, *Biochem. J.* **42**, 35 (1948).
24. C. H. Gallagher, *Biochem. J.* **74**, 38 (1960).
25. T. H. Huismann, *J. Acta endocrinol.* **13**, 55 (1953).