are taken into consideration: morphine is known to have striking depressive effects on the blood circulation and on respiration, resulting in a certain degree of anoxia.

In rats with intra-aortic cannulas, we have noted that morphine leads to a 30% decrease in blood O_2 concentration and to a simultaneous increase of 20% in the CO_2 concentration. Moreover, some tissues incubated in vitro show an enhanced uptake of glucose without increased lactate production when morphine is added to the medium[4,5], very probably because there is no deficiency of oxygen in this medium. However, it is unlikely that the in vivo lactate excess results exclusively from cellular hypoxia. When the depressive effects of the drug on respiration are inhibited by nalorphine, lactate accumulation persists, but is reduced. A study on rats[6] shows nalorphine alone has effects as marked as those of morphine; but in the rats receiving both drugs simultaneously, the antagonism of nalorphine towards the respiratory depressive effect of morphine is evident. Under our experimental conditions, the lactate accumulation was only partly reduced. It is necessary to search for other causes of the high plasma lactate. It is possible that an increased secretion of adrenaline due to morphine administration is involved. Many studies on morphine-induced hyperglycemia have implicated this hypersecretion. We have conducted some additional experiments at the histochemical level[7] and found that morphine causes a brief but marked depletion of the granules of the adrenal medulla. Moreover preliminary experiments on rabbits show that the simultaneous administration of β-blockers (butoxamine, 25 mg/kg) completely inhibits the rise in plasma lactic acid level normally induced by morphine. In such a case, the increase of plasma lactate can reasonably be attributed to the stimulation of the sympathetic nervous system.

Although the effect of the first morphine doses can easily be explained, the situation in chronically treated rats is less clear. In these animals, development of a tolerance towards the immediate effect of the drug occured which corresponded to a gradual decrease in the activity in the adrenal medulla. Successive injections of morphine did not cause the depletion of pheochromic granules found with the first injections. In chronically intoxicated and in abstinent animals, plasma lactic acid concentration remained abnormally high, even 1 week after withdrawal. The adrenal medulla remained in an inhibited state but the blood sugar and the oxygen consumption were normal. Thus, a relationship between the high concentration of blood lactic acid and the secretory activity of the adrenal medulla was not seen, perhaps because the regulation of cellular metabolism is profoundly modified. For example, in vivo glycolysis of various organs of chronic morphine-treated animals was found to be significantly increased[8].

The increase of lactate production seems to be a symptom of a deep and lasting disturbance in cellular metabolism induced by morphine intoxication. Whatever the origin, the lactate excess is interesting to consider in cerebral tissue in which the biochemical composition is generally very stable. As a result of the lactate excess, disorders occur in the central nervous system, and these disorders might be related to the typical phenomena of tolerance and physical dependence.

Résumé. Chez le rat, une première dose de morphine (20 mg/kg) entraîne une hausse du taux de l'acide lactique du sang et du cortex cérébral. Si les injections sont répétées la tolérance se développe vis-à-vis de cet effet immédiat et de plus, les animaux chroniquement traités possèdent un taux de base d'acide lactique anormalement élevé. Cette anomalie persiste au moins une semaine après sevrage.

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Glycogen Metabolism and the Effect of Nitroglycerin on the Glycogen Metabolism in the Normal and Ischemic Canine Myocardium

Ischemia or anoxia of the heart causes acceleration of glycogenolysis and accumulation of the intermediates of glycolysis in the myocardium[1-6]. These studies were conducted on the assumption that the heart muscle must be homogeneous from the view point of metabolism. Jedeikin[7], however, reported that the level of glycogen and activity of glycogen phosphorylase in the endocardial layers were higher than those in the epicardial layers. This suggests that there are some differences in metabolism between the endo- and epicardial layers, especially when the heart is ischemic. The present study was conducted in an attempt to examine this possibility, and to study the effect of nitroglycerin on the myocardial metabolism.

Mongrel dogs anesthetized with pentobarbital were used. Under artificial respiration, one of the small branches of the coronary artery was ligated 5 min after i.v. injection of saline or nitroglycerin. Just before or after ligation of the small branch (1.5, 3, 7 and 30 min after the ligation), the heart was removed, and immediately frozen with freezing clamps. The endo- and epicardial portions of the left ventricle, which had been nourished by the small branch, were taken for determination of glycogen, glucose-6-phosphate (G6P), lactate, adenosinetriphosphate (ATP) and phosphocreatine (PCr), and activities of phosphorylase *a* and *b*. Results are shown in the Figure and the Table.

Results. 1. *Control (saline-injected) dogs.* In non-ischemic hearts, the levels of glycogen, G6P and lactate, and

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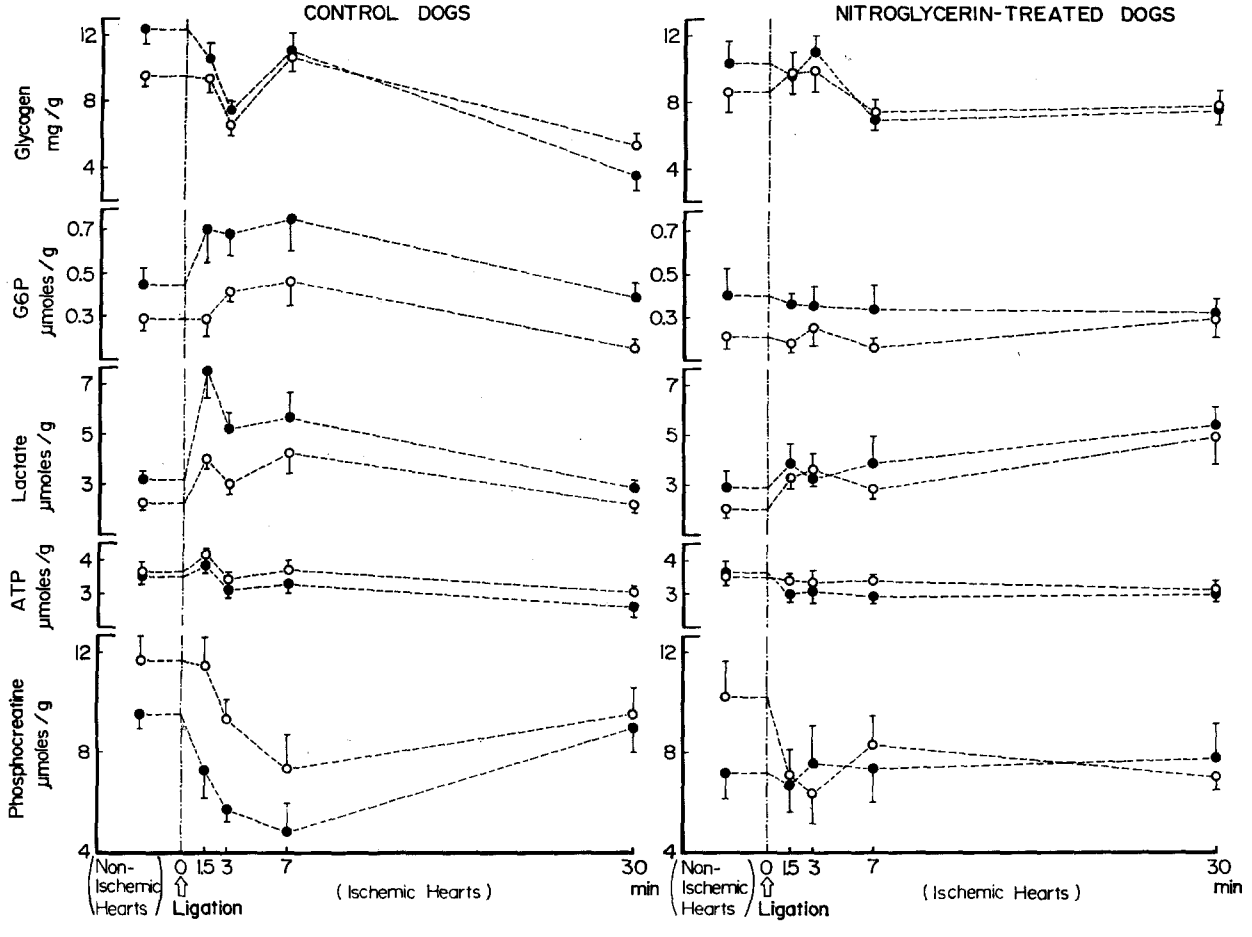
The activities of phosphorylase in the normal and ischemic hearts and the effect of nitroglycerin on these activities (μmoles Pi released/g-wet tissue/min)

		Phosphorylase *a* (without AMP)		Phosphorylase *a* + *b* (with AMP)		
		Endocardial layers	Epicardial layers	Endocardial layers	Epicardial layers	
Control dogs						
Time after ligation						
Before	(8)	5.0 ± 0.5	3.2 ± 0.4	16.6 ± 0.5	12.6 ± 0.3	(Non-ischemic)
1.5 min	(7)	8.3 ± 1.4	7.9 ± 1.3	16.8 ± 1.7	12.7 ± 1.4	
3	(8)	9.3 ± 1.0	7.1 ± 1.2	15.8 ± 1.4	12.9 ± 1.4	(Ischemic)
7	(7)	6.0 ± 0.4	3.9 ± 0.3	16.4 ± 0.7	11.9 ± 0.8	
30	(6)	4.7 ± 0.3	2.8 ± 0.6	14.9 ± 1.1	11.3 ± 1.2	
Nitroglycerin-treated dogs						
Time after ligation						
Before	(8)	6.0 ± 1.2	5.6 ± 0.7	16.2 ± 1.1	11.5 ± 1.4	(Non-ischemic)
1.5 min	(8)	6.4 ± 0.8	4.8 ± 0.7	13.5 ± 1.1	12.7 ± 1.2	
3	(8)	5.6 ± 0.7	4.8 ± 0.7	15.9 ± 1.7	12·0 ± 1.4	(Ischemic)
7	(8)	5.8 ± 0.7	4.8 ± 0.8	16.2 ± 1.3	12.5 ± 0.9	
30	(8)	6.1 ± 0.6	5.0 ± 0.5	15.6 ± 0.9	15.0 ± 1.1	

Values are mean ± S.E.M. Number of animals in parenthesis



The effect of ligation of one of the small branches of the anterior descending coronary artery on the endocardial (solid circle) and epicardial (open circle) levels of glycogen, G6P, lactate, ATP and phosphocreatine in control (saline-injected) dogs and nitroglycerin-treated dogs. The small branch was ligated 5 min after the injection. The dose of nitroglycerin injected i.v. is 20 μg/kg. 'Non-ischemic hearts' represent the hearts removed just before the ligation, and 'ischemic hearts' represent the hearts removed after the ligation. Each point with a bar represents mean ± S.E.M. of 6 to 8 hearts. It should be noted that there is a difference between the control dogs and the nitroglycerin-treated dogs in the pattern of the process of anaerobic metabolism.

activities of phosphorylase *a* and phosphorylase *a* + *b* in the endocardial layers were higher ($p < 0.01$) than those in the epicardial layers. The PCr level of the endocardial layers, however, was lower ($p < 0.05$) than that of the epicardial layers. No significant differences were detected in the ATP level between the endo- and epicardial layers. The ligation of the small branch resulted in a marked decrease in glycogen level in each of the two layers, and caused a conversion of phosphorylase from *b* form to *a* form. The rate of decrease in glycogen level after the ligation in the endocardial layers was faster than that in the epicardial layers. The level of G6P and of lactate in each of the two layers increased rapidly after the ligation. The level of ATP in each of the two layers was not appreciably altered by the ligation. The PCr level in each of the two layers decreased markedly until at least 7 min after the ligation. The rate of decrease in the endocardial PCr level was more rapid than that in the epicardial PCr.

2. *Nitroglycerin-treated dogs.* Nitroglycerin (20 µg/kg) was injected instead of saline in this series of experiments. In non-ischemic hearts, the levels of glycogen, G6P, lactate and ATP, and the activities of phosphorylase *a* and phosphorylase *a* + *b* in each of the two layers did not differ from those obtained in control dogs. The level of PCr in each of the two layers, however, was slightly lower than that obtained in control dogs. Nevertheless, the level of PCr in the endocardial layers was always lower than that in the epicardial layers. The ligation of small branch of coronary artery produced neither marked decrease in glycogen level nor rapid conversion of phosphorylase from *b* form to *a* form in both the layers. The level of ATP in each of the two layers was not altered by the ligation. After the ligation, the level of PCr in the epicardial layers decreased slightly, but that in the endocardial layers did not.

Zusammenfassung. Nachweis, dass die Unterbindung schon eines kleinen Astes der Koronararterie des Hundes im Endokard eine stärkere Steigerung der anaeroben Glykolyse als im Epikard bewirkt. Vorbehandlung mit Nitroglycerin hemmt die Steigerung der durch die Ligatur bedingten anaeroben Glykolyse.

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Interaction of Drugs: A Mathematical Model and its Application in Bacteriology

In a previous paper[1] it was reported that novobiocin combined with tetracycline had a synergistic bactericidal effect on *Pseudomonas pseudomallei*. This communication centres on the analysis of the mathematical model that has been put forward to represent the relation between response and dose of drugs, alone and in combination, and on the graphic presentation of the values indicated in the model. Two combinations only are presented here, the novobiocin-tetracycline and the kanamycin-chloramphenicol; the former is bactericidal on *P. pseudomallei*, strain 6[1], the latter on *Escherichia coli*, strain 80[1].

Mathematical model. The concept of additivity of drugs is predicated by the following model. Let N denote the number of bacteria present at time t (in h), and N_0 the number of bacteria present originally ($t = 0$). In the absence of drugs, it is assumed that bacteria grow at a rate proportional to the amount present $\frac{dN}{dt} = kN$ where k denotes the growth rate. The rate of bactericidal activity of drugs, single or in combination, is proportional to the number of bacteria present and the amount of drug(s) used:

$$\frac{dN}{dt} = kN - czN = -(cz - k)N \qquad (1)$$

where z denotes the amount of drug(s), single or mixed in fixed proportion, and c is a constant which denotes the specific bactericidal activity of drugs, single or in combination. By solving equation (1), we have

$$N = N_0 e^{-(cz-k)t}$$

and, after a fixed time interval t_1 (24 h, e.g.), the corresponding bacterial count N_1 satisfies the equation

$$\frac{N_0}{N_1} = \frac{1}{e^{-(cz-k)t_1}} = e^{(cz-k)t_1}. \qquad (2)$$

We define the log response q to a drug dose as

$$\log_{10}\frac{N_0}{N_1}.$$

This quantity represents conveniently, on a decreaings scale, the number of viable bacteria in function of the dose tested at time $t_1 = 24$ h:

$$q = \log_{10}\frac{N_0}{N_1} = \log_{10}[e^{(cz-k)t_1}] = (cz-k)\,t_1 \log_{10} e = 0.434\,(cz-k)\,t_1. \qquad (3)$$

According to this mathematical model, the log response will vary linearly with the total amount z of drug(s) tested, single or mixed in fixed proportion. Since

$$q = [(0.434\,c)\,z - 0.434\,k]\,t = (c'z - k')\,t \qquad (4)$$

the specific rate of bactericidal activity c' depends only on the amount and proportion of drugs tested z while the rate of growth k' depends only on the strain of bacteria used.

Let us suppose that several drugs D_1, D_2, D_3 ... that have bactericidal rates c'_1, c'_2, c'_3 ... are tested in combination in the following amounts, respectively, z_1, z_2, z_3 ... We shall say that the action is additive if eq. (1) becomes

$$\frac{dN}{dt} = kN - (c_1 z_1 + c_2 z_2 + c_3 z_3 \ldots)\,N$$

so that

$$q = \log_{10}\frac{N_0}{N_1} = 0.434\,(-k + \sum_n c'_n z_n)\,t_1 = (\sum_n c'_n z_n - k')\,t. \qquad (5)$$

When two drugs are tested in combination, then

$$q = (c'_1 z_1 + c'_2 z_2)\,t_1 - k'\,t_1. \qquad (6)$$

A given pair of drugs exhibits isobole additivity at a particular level of response q, if the corresponding isobole is rectilinear. If the isobole is either markedly convex or concave toward the origin, the combined effect is said to be synergistic in the first case, and antagonistic in the

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