purine nucleotide cycle can explain early experiments with nitrogen-15 ammonia which showed that there is a rapid turnover of the 6-amino group of adenine mononucleotides of striated muscle in vivo, in contrast to a very slow turnover of the ring nitrogens (*14*).

The probable functions of the purine nucleotide cycle fall into several overlapping categories. (i) It is a pathway for the liberation of ammonia from amino acids. Unlike ammonia liberation by way of the glutamate dehydrogenase reaction, ammonia liberation by way of the purine nucleotide cycle is energetically favored. Moreover, muscle either lacks glutamate dehydrogenase or possesses very low amounts of this enzyme. (ii) It is a pathway for the adjustment of the concentrations of citric acid cycle intermediates. Muscle and most other tissues lack pyruvate carboxylase. Malic enzyme has kinetic characteristics that make a net synthesis of malate from pyruvate very unfavorable under most physiological conditions. The aspartate aminotransferase reaction does not lead to a net production of oxaloacetate and α-ketoglutarate. The purine nucleotide cycle provides an alternative pathway for the net supply of four-carbon, dicarboxylic acids to the citric acid cycle. (iii) It is a pathway for regulating the relative concentrations of the adenine nucleotides AMP, ADP, and ATP. Deamination of AMP to IMP leads to a readjustment of the myokinase equilibrium (*15*) (reaction 4)

$$2\ \text{ADP} \longleftrightarrow \text{AMP} + \text{ATP} \qquad (4)$$

toward ATP, with a resulting increase in the concentration ratio of [ATP] to [ADP]. This type of adjustment may be necessary when the resynthesis of ATP from ADP by other pathways is inadequate. (iv) It is a pathway which aids in the control of phosphofructokinase activity. Ammonium ions stimulate phosphofructokinase. In addition, the liberation of ammonia from AMP serves to raise the intracellular *p*H. Inhibition of phosphofructokinase by physiological concentrations of ATP is very pronounced below *p*H 7.1. It is negligible above *p*H 7.3. Full details and documentation for these functions are provided elsewhere (*16*).

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Cyclic Adenosine and Guanosine Monophosphates and Glucagon: Effect on Liver Membrane Potentials

Abstract. *Cyclic adenosine monophosphate, cyclic guanosine monophosphate, glucagon, and isoproterenol each hyperpolarized perfused rat liver cells. The hyperpolarization followed a time course similar to the stimulated increase in potassium efflux and was preceded by the increase in calcium efflux. The hyperpolarization induced by cyclic adenosine monophosphate was blocked by tetracaine. The similarity of the action of the cyclic nucleotides to that of glucagon supports the hypothesis that cyclic adenosine monophosphate is the secondary messenger mediating the action of glucagon.*

Adenosine 3′,5′-monophosphate (cyclic AMP) is thought to be the secondary messenger mediating the action of epinephrine and glucagon on glycogenolysis, gluconeogenesis (*1, 2*), calcium efflux (*3, 4*), and transmembrane potassium flux in the rat liver (*3, 5*). Cyclic AMP also mediates the potassium-dependent beta-adrenergic hyperpolarization in vascular smooth muscle (*6*), and the inhibitory action of catecholamines on the discharge frequency, membrane potential, and membrane resistance of rat cerebellar Purkinje cells (*7, 8*). Guanosine 3′,5′-monophosphate (cyclic GMP) is another naturally occurring nucleotide that has effects similar to those of cyclic AMP on the carbohydrate metabolism of the liver (*9*). The purpose of our study was to determine whether cyclic AMP, cyclic GMP, and the agents that increase endogenous hepatic cyclic AMP concentrations, such as glucagon and the beta-adrenergic agent isoproterenol (*1*), have an effect on the membrane potential of the liver cells. Furthermore, as an effect on the membrane potential became evident, we correlated this with the time course of the ion fluxes induced in the liver by glucagon and cyclic AMP. Finally, since tetracaine, a local anesthetic, blocks the calcium efflux, glycogenolysis, and gluconeogenesis induced by glucagon and cyclic AMP (*4*), we proceeded to establish a similar inhibitory action on the hyperpolarizing effects of cyclic AMP.

Rat livers were perfused in situ with Krebs' bicarbonate solution containing 4 percent albumin and 20 m*M* sodium pyruvate (*4, 10*). Membrane potentials were determined with floating intracellular microelectrodes (*11*), and prior to the addition of drugs were greater (Table 1) than those reported in liver slices, but within the range or slightly below those measured in nonperfused animals (*12*).

Cyclic AMP (1 m*M*), cyclic GMP (0.5 m*M*), or glucagon (0.1 μg/ml) produced significant hyperpolarization (Table 1) after a delay (*13*) of 4 to 8 minutes (Fig. 1). Isoproterenol (0.2 μg/ml) had a similar hyperpolarizing effect that was not abolished by the alpha-blocker, phentolamine (Fig. 1). Isoproterenol also increases the cyclic AMP concentration in the liver (*14*). Adenosine 5′-monophosphate (1 m*M*) in four experiments had no effect on the membrane potential and in another four rats it produced a slight hyper-

polarization (3, 4, 5, and 7 mv). For the entire series of eight animals, this effect was not significant ($P > .1$).

The hyperpolarizing action of cyclic AMP was completely abolished by tetracaine (0.5 m*M*). Tetracaine itself produced a very transient hyperpolarization followed by depolarization and preceded by a slight depolarization (Fig. 1).

Cyclic AMP and also glucagon produce an early increase in calcium efflux from the perfused rat liver (*3, 4*), followed by the increase in potassium efflux (*3, 4*). Since the previously reported experiments were performed at 37°C, some of these were repeated at temperatures similar to those used for the membrane potential studies in order to correlate the ion fluxes with changes in membrane potential. Even at 27°C the increase in calcium efflux precedes the hyperpolarization produced by cyclic nucleotides and by glucagon. Cyclic GMP (0.5 m*M*) in similar experiments also increased the potassium concentration (by 1 ± 0.2 m*M*; $n = 4$) of the effluent. The time course of the stimulated potassium efflux is very similar to that of the hyperpolarization, suggesting that the two events are related. Delayed stimulation of potassium efflux is also produced by beta-adrenergic amines in the myometrium (*15*) and by cyclic AMP and catecholamines in fat cells (*16*). Inasmuch as the potassium equilibrium potential of the liver cell is more negative than the resting potential measured (*17*), a delayed increase in potassium permeability with the resultant net outward potassium current could be responsible for the hyperpolarization observed. The possibility, however, that the hyperpolarization reflects the activity of an electrogenic cation pump (*18*) cannot be eliminated without membrane resistance measurements. In rat cerebellar Purkinje cells the hyperpolarizing action of cyclic AMP is associated with an increase in membrane resistance (*8*) strongly negating the possibility of it being mediated by a passive increase in potassium permeability of the membrane. Although, in the guinea pig liver, catecholamines produce an immediate hyperpolarization associated with a decrease in membrane resistance probably due to an early increase in potassium permeability, this is an alpha-adrenergic effect, not a beta-adrenergic one (*17*). There are marked species differences in the behavior of adrenergic receptors of the liver (*2, 19*), hence it is possible that beta-adrenergic responses (mediated by increased amounts of cyclic AMP) are less pronounced in the guinea pig than in the rat or, if present, are delayed beyond the time of the membrane potential observations reported. Finally, inward anion (chloride) in excess of the outward cation (K^+) movement could theoretically account for the hyperpolarization observed. This possibility has thus far been tested only in vascular smooth muscle (*20*), where it was found that substitution of extracellular chloride with the impermeant

Table 1. Hyperpolarizing responses of perfused rat liver cells. Each number represents the mean membrane potential (mv) and standard error of approximately 15 penetrations. The membrane potentials showing the drug effects were averaged for the period of hyperpolarization determined from plots similar to Fig. 1.

Substance	Control (mv ± S.E.)	After drug* (mv ± S.E.)
3′,5′-AMP (1.0 m*M*)	30 ± 0.6	38 ± 0.7
	27 ± 1.1	33 ± 0.8
	28 ± 0.7	38 ± 0.8
	33 ± 0.7	46 ± 1.5
	39 ± 1.0	46 ± 0.9
3′,5′-GMP (0.5 m*M*)	34 ± 0.6	45 ± 0.7
	36 ± 0.6	47 ± 0.6
	40 ± 0.5	48 ± 0.8
Glucagon (0.1 μg/ml)	36 ± 1.0	45 ± 1.2
	42 ± 0.6	51 ± 1.8
	33 ± 0.6	46 ± 2.0
	37 ± 0.5	49 ± 0.9
Isoproterenol	35 ± 0.8	43 ± 1.3
	41 ± 1.0	50 ± 1.7
	37 ± 0.5†	46 ± 0.8
	38 ± 0.8†	44 ± 1.3

* The difference between each pair was highly significant ($P < .001$). † 1.0 μg of phentolamine hydrochloride per milligram was present.

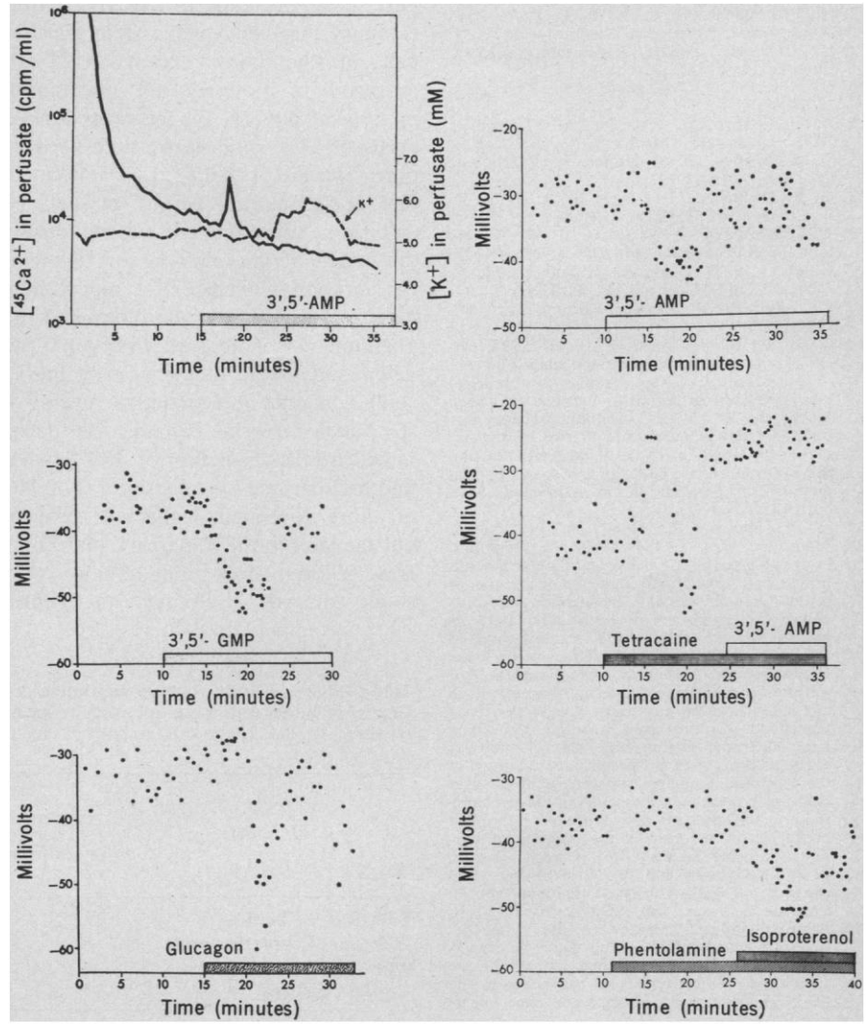

Fig. 1. Membrane potential and ion fluxes in perfused rat liver. The potassium efflux curve (top left panel) is the mean of three experiments. Bars (abscissa) indicate the duration of drug action. In the $^{45}Ca^{2+}$ (solid line, top left panel) and K^+ flux experiments, the cyclic AMP was added at a different point of the abscissa (plotted at 15 minutes after 0 time) whereas in the electrophysiological experiments it was added at 10 minutes.

anion isethionate (2-hydroxyethanesulfonate) did not decrease the hyperpolarizing action of either isoproterenol or dibutyryl cyclic AMP. Therefore, at least in smooth muscle, beta-adrenergic hyperpolarization is not due to inward chloride movement.

Our findings raise the possibility that changes in membrane potential produced by cyclic AMP may interact with, and possibly modulate, metabolic processes in the liver.

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Artibeus jamaicensis: Delayed Embryonic Development in a Neotropical Bat

Abstract. *In Panama the phyllostomid bat* Artibeus jamaicensis *is seasonally polyestrous, and young are born in March or April and July or August. Blastocysts conceived after the second birth implant in the uterus but are dormant from September to mid-November, when normal development again resumes.*

A recent investigation of the breeding patterns of Central American bats (*1*) indicated that the frugivorous bat *Artibeus jamaicensis* (family Phyllostomidae) is polyestrous and that, in Panama at least, it has birth peaks in March through April and in July through August, near the end of the dry season (January through April) and in the first half of the rainy season. Although polyestry is probably not uncommon in tropical bats (*1*, *2*), the reproductive cycle of *A. jamaicensis*, and perhaps other species of *Artibeus*, is unique in that a 2.5-month period of delayed embryonic development occurs during the height of the rainy season (September through November). I report here details of the early embryology and the phenomenon of delayed embryonic development in the Jamaican fruit bat.

The annual reproductive cycle of *A. jamaicensis* in Panama, as determined by the dissection of 450 females and microscopic examination (*3*) of 167 of those specimens collected throughout the year in the Panama Canal Zone, is as follows. Most adult females carry single embryos in January and February and give birth in March or April. A postpartum estrus then occurs, as indicated by several lactating specimens caught in March. (The uteri of these specimens were still enlarged and filled with debris but they contained sperm, and each specimen had a newly forming corpus luteum in one ovary.) Females may be simultaneously pregnant and lactating in March, April, or May. After a gestation period of no more than 4 months, the second young is born in July or August. A postpartum estrus may also occur after this birth, although direct evidence for this is provided by only two sectioned specimens. Blastocysts from this fertilization apparently implant in the uterus in late August or early September but do not begin continuous development until mid-November. These embryos become macroscopically visible in December and are born in March or April.

Several features of implantation and early embryology in *A. jamaicensis* are similar to those of the neotropical bats *Glossophaga soricina* (family Phyllostomidae) and *Desmodus rotundus* (family Desmodontidae) (*4*, *5*). These include

Table 1. Size of corpus luteum and lutein cells in pregnant individuals of *A. jamaicensis*. Mean lengths of lutein cells were obtained by measuring four lutein cells per corpus luteum wherever possible. In the lutein cell column *N* indicates the actual number of cells measured; S.E., standard error.

Type of female	Corpus luteum			Lutein cells		
	N	Mean diameter (μm)	S.E.	*N*	Mean length (μm)	S.E.
With delayed blastocyst	19	1803.16	68.02	96	33.53	0.972
With nondelayed blastocyst	5	1918.00		26	29.81	0.980
With macroscopically visible embryo	12	2284.58	86.62	44	38.47	1.268