turn over independently of each other. Further, the mechanism of increase for each constituent after phenobarbital administration would appear to be different; the protein portions showing solely an increase in synthesis without any decrease in catabolism, while the phospholipid portions show primarily a decrease in catabolism with little or no increase in synthesis.

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Adenyl cyclase in non-nucleated erythrocytes of several mammalian species

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EARLY reports to the effect that the nucleated avian erythrocytes, but not the non-nucleated canine variety, possessed measurable adenyl cyclase activity[1] lead to a generalization that only nucleated cells possessed this enzyme. A subsequent report that the erythrocytes of frogs and tadpoles contained an adenyl cyclase stimulated by catecholamine and fluoride while those of humans were devoid of a catecholamine-stimulated enzyme[2] supported this concept. However, the dog erythrocyte is deficient in a sodium pump and thus probably a Na^+-K^+ activated ATP-ase, making it atypical of non-nucleated erythrocytes in this respect. One must also consider that the absence of a catecholamine-stimulated adenyl cyclase in human erythrocytes does not preclude the presence of an enzyme which is stimulated by fluoride or other hormones.

The enzyme is reported to be present in the plasma membrane,[2,3] and, therefore, non-nucleated erythrocytes would yield a membrane preparation free of contamination from other cellular inclusions. Motivated by a desire to study the activation and inhibition of adenyl cyclase a decision was made to investigate the possibility that non-nucleated erythrocytes of certain species possessed a hormone-activated enzyme. It was also of interest to examine the possible positive correlation of adenyl cyclase with a sodium pump. Therefore, the high Na^+-containing erythrocytes from the cat and dog were compared with the high K^+-containing erythrocytes of the human, rat and mouse.

The blood from the human, cat and dog was collected in heparinized syringes while that from the mouse and rat was collected in heparinized test tubes after decapitation. The red blood cells were centrifuged at 1000 rpm, washed with 1 vol. of 322 milliosmolar-phosphate buffer, recentrifuged, washed as above and finally centrifuged at 2000 rpm. All of these steps were carried out at 5°. Two ml of packed cells was diluted with 26 ml of hypotonic (20 milliosmolar) NaCl-phosphate buffer, mixed with a Vortex mixer and centrifuged at 20,000 *g* for 40 min at 5° in a Beckman L-2 ultracentrifuge. The supernatant was removed with a syringe and the ghosts reconstituted to the original volume of cells.

Adenyl cyclase activity was determined by incubating 0·2 ml of ghosts with $MgCl_2$ (1 μmole), adenosine-3′, 5′-cyclic phosphate (1 μmole), 1 μc ATP-8-^{14}C (0·24 μmole) in a total of 0·5 ml of 0·04 M Tris-HCl buffer (pH 7·4) for 30 min at 37°.[4] The reaction was stopped by placing the vessels in boiling water for 3 min. The samples were centrifuged at 3000 rpm for 10 min. A 0·1-ml aliquot of the supernatant was added to 6 λ of a solution containing ATP, ADP, AMP, adenosine and adenine each at a concentration of 10^{-2}M. This solution was evaporated to dryness with N_2 and the residue was dissolved in 60 λ of 50% ethanol. This was placed on Whatman No. 1 filter paper and chromatographed overnight in an ascending fashion with solvent A composed of absolute ethanol: 1 M NH_4Ac: H_2O (5:1:1). The adenosine-3′, 5′-cyclic phosphate (cyclic-AMP) spot was detected by absorption in ultraviolet light, cut out and counted in the liquid scintillation counter in a system composed of 10 cm^3 of toluene containing 0·4% butyl-PBD. The ATP-ADP-AMP area was likewise counted in order to determine the per cent of total ^{14}C in the cyclic-AMP area. No significant breakdown of this amount of C-AMP was noted during the incubation. The areas just counted were replaced in their appropriate spaces of the chromatogram and autoradiograms were prepared with Royal Blue X-Ray film over a 56 hr period. This permitted one to evaluate possible contamination from overlapping spots and only that radioactivity which conformed to the shape of the cyclic-AMP spot was considered valid. The identity of the cyclic-AMP was further supported by the position of the ^{14}C in solvent system B containing isobutyric acid:H_2O:concentrated NH_3 (66:33:1), its non-precipitability with $Ba(OH)_2$ and Zn SO_4[5] and its convertibility to AMP by a soluble phosphodiesterase preparation with no alteration of specific activity.

TABLE 1. ACTIVITY OF ADENYL CYCLASE OF ERYTHROCYTE GHOSTS FROM SEVERAL MAMMALIAN SPECIES*

	nmoles of Cyclic-AMP produced/incubation		
Species*	Control	NE (10^{-3}M)	NaF (10^{-2}M)
Human	0·031 ± 0·003	0·029 ± 0·004	0·085 ± 0·010‡
Cat	0·043 ± 0·003	0·043 ± 0·004	0·074 ± 0·008†
Dog	0·044 ± 0·009	0·036 ± 0·004	0·108 ± 0·008‡
Mouse	0·040 ± 0·004	0·262 ± 0·021‡	1·196 ± 0·076†
Rat	0·044 ± 0·008	0·249 ± 0·029‡	0·715 ± 0·040‡

* The data were obtained from duplicate analyses of three separate samples of blood. In the case of the rat and mouse, each sample was a pool from two and thirteen individuals, respectively. P values relative to control incubations: † < 0·01, ‡ < 0·001.

The results demonstrate that of the five species tested only the rat and the mouse erythrocytes possessed adenyl cyclase activity which was stimulated by 10^{-3}M norepinephrine (NE). The adenyl cyclase of these two species was stimulated even more in the presence of 10^{-2}M NaF. The very slight increase in the cyclic-AMP-14C areas of the human, cat and dog erythrocytes incubated with NaF was statistically significant but may not all be due to cyclic-AMP. In the presence of NaF these three species produce significant amounts of ^{14}C which migrate just forward of the cyclic-AMP area and may have contributed to the counts in this area. This material, like xanthine, moves slower in solvent B but can be separated from carrier xanthine. Its identity, therefore, remains unknown.

The probability that the activity found in the ghost preparations could have resulted from platelet

or leukocyte contamination must be considered. Fluoride stimulation resulted in an increase in the production of cyclic-AMP by the cells of the buffy coat of the cat (1·3 nmoles/mg protein), mouse (3·7 nmoles/mg protein) and rat (4·0 nmoles/mg protein) as compared with that of the erythrocyte ghosts of the mouse (0·38 nmoles/mg protein) and the rat (0·18 nmoles/mg protein). With rat platelets, fluoride stimulation of adenyl cyclase resulted in C-AMP production of 1·2 nmoles/mg protein. It is clear that the specific activity of the leukocyte and platelet adenyl cyclase is greater than that of the erythrocyte ghosts. It should be noted, however, that no norepinephrine stimulation could be detected with leukocyte and platelet preparations suggesting that these cells are responsive to some other hormones. In addition, microscopic examination of the erythrocyte preparations demonstrated that while platelet clumps with some trapped leukocytes were visible in washed erythrocyte fractions, none were visible in the ghost preparation. Instead, one obtains a pellet which possesses a fluoride but not norepinephrine-stimulated adenyl cyclase and is largely composed of platelets with some trapped leukocytes. We can safely say, therefore, that the fluoride and especially the norepinephrine-stimulated adenyl cyclase of the rat and mouse erythrocyte ghosts preparations reside in the plasma membranes of the erythrocytes and are not derived from contaminating platelets or leukocytes.

It is clear, therefore, that under the conditions of these experiments both NE- and NaF-stimulated adenyl cyclase can be found in non-nucleated erythrocytes such as the mouse and rat. Both of these species have an active sodium pump but the barely detectable adenyl cyclase in the human, cat and dog makes it impossible to support the concept that the presence of this enzyme might be correlated with that of an active sodium pump.

It remains to be demonstrated whether the adenyl cyclase in rat and mouse erythrocytes has an active role to play or only represents a residue from a functional past. Further characterization of the enzyme of the rat erythrocyte is underway.

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l-Asparaginase resistance in human leukemia—Asparagine synthetase

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l-ASPARAGINASE inhibition of rodent tumors has been related to *l*-asparagine depletion of tumor cells that require an exogenous source of *l*-asparagine.[1,3] It has since been demonstrated that a mechanism of *l*-asparaginase resistance in nonsensitive murine tumors is the presence of an *l*-asparagine synthetic pathway, mediated by the enzyme asparagine synthetase.[4,10] Studies on the control of *l*-asparagine biosynthesis in mammals have shown product inhibition of asparagine synthetase, a lack of substrate stimulation of asparagine synthetase by NH_4Cl or *l*-aspartic acid, and markedly increased asparagine