stable bioantioxidants lose their activity. It was shown (Fig. 1) that the character of autoacceleration and the level of oxidation at which escape from the induction period takes place may be determined both by α-tocopherol in concentrations of over $2.0 \cdot 10^{-3}$ M and by mixtures of α-tocopherol with phospholipids (400-800 mg %) in concentrations characteristic of native lipids ($0.8 \cdot 10^{-3}$-$1.2 \cdot 10^{-3}$ M).

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EFFECT OF ADENOSINE, AMP, AND PAPAVERINE ON THE cAMP CONTENT IN [^{14}C]ADENINE PRELABELED THYMOCYTES

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Adenosine has a toxic action on lymphocytes [2, 12], but the mechanism of this action has not been explained. According to one hypothesis, this nucleoside affects lymphocyte function through the adenylate cyclase system, by increasing the intracellular cAMP concentration [4, 8, 14]. AMP can influence the cAMP level in lymphocytes *in vivo* more effectively still because of its higher concentration in the blood plasma than that of adenosine, and coupling of the action of 5'-nucleotidase and adenylate cyclase in the plasma membrane [6]. Papaverine also raises the cAMP concentration in cells [13] and inhibits blast transformation of thymus lymphocytes induced by concanavalin A [2]. The effectiveness and direction of action of cAMP on immune function are not always the same [3], possibly depending on the existence of special cAMP compartments, under the control of different effectors, in lymphocytes [9].

In the investigation described below the effect of various factors (adenosine, AMP, and papaverine) on the cAMP concentration was studied in thymocytes prelabeled with [^{14}C]adenine.

EXPERIMENTAL METHOD

Wistar rats aged 1.5-2 months were used. Thymocytes were isolated and then incubated in Hanks' medium, buffered with 20 mM HEPES and $NaHCO_3$ to pH 7.4. Thymocytes were labeled with [^{14}C]adenine (specific radioactivity 20 mCi/mmole) in a plastic vessel. The incubation (1 h at 37°C) the cells were washed twice and resuspended in cold isolation medium at 4°C. The incubation medium contained $3 \cdot 10^7$ cells in 1 ml and 25 μM of labeled adenine. After incubation (1 h at 37°C) the cells were washed twice and resuspended in cold isolation medium at 4°C. The resuspension was poured into test tubes in volumes of 15 ml, and after preincubation for 10 min at 37°C, Hanks' solution (control) and the test substances were added to them so that the final concentration of the latter was 100 μM and of cells $2 \cdot 10^7$/ml. After incu-

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TABLE 1. Effect of Test Substances on Content and Specific Radioactivity of cAMP and ATP in Thymocytes (M ± m, n = 6)

Substance	Total content, pmoles/10^7 cells		Specific radioactivity, cpm/pmole	
	cAMP	ATP	cAMP	ATP
control	1,65 ± 0,37	1557 ± 8,9	38,2 ± 3,3	12,0 ± 0,74
Papaverine	3,2 ± 0,21	942 ± 166*	46,2 ± 4,4*	6,4 ± 0,82*
Adenosine	3,4 ± 0,4	2570 ± 91*	24,7 ± 3,9*	7,3 ± 0,56*
ATP	3,1 ± 0,24	2495 ± 279*	26,0 ± 4,1*	7,1 ± 0,59*
Papaverine + AMP	9,7 ± 1,1	965 ± 120*	20,5 ± 2,9*	5,9 ± 0,68*
Papaverine + adenosine AMP	8,9 ± 0,9	952 ± 31*	19,0 ± 2,4*	6,0 ± 0,63*

Legend. *P = 0.05 compared with control.

bation for 20 min at 37°C the thymocytes were sedimented by centrifugation (1500g, 1 min). The residues were resuspended in 1 ml of 6% $HClO_4$ and, to extract the nucleotides more completely, allowed to stand overnight at 4°C. After deproteinization and neutralization with KOH the perchlorate extracts were adjusted to a final volume of 2 ml. To determine ratioactive cAMP, 500 µl of the extract was treated with $ZnSO_4$ and $Ba(OH)_2$ solutions [1] and 450 µl was applied to chromatography paper together with unlabeled cAMP in a strip of suitable length for this volume. Chromatography was carried out in 1 M ammonium acetate and 97% ethanol (15:35). Adenine nucleotides also were fractionated by paper chromatography [7], using 50 µl of the perchlorate extracts with appropriate reference substances. After chromatography, stains visible on the paper under UV light were cut out and immersed in vessels wih ZhS-1 scintillation fluid for measurement of their radioactivity. The total content of cAMP in the perchlorate extracts was determined by means of a kit from Amersham Corporation (England). Radioactivity of protein-bound [^{3}H]-cAMP was measured on the third channel of the counter. Under these circumstances, radioactivity due to ^{14}C was negligible and was disregarded. The content of adenine nucleotides was determined in fractions obtained after chromatography of 500 µl of the perchlorate extracts on columns with Dowex 18 resin in the H^+ form, by a fluorescence method [2]. Adenylate cyclase activity was determined as described previously [1].

EXPERIMENTAL RESULTS

Specific radioactivity of cAMP in thymocytes prelabeled with [^{14}C]adenine was 2.6 times greater than that of ATP (Table 1). Considering these data and also the fact that the sole source for cAMP biosynthesis and ATP, it can be concluded that the thymocytes possess a special ATP compartment destined for the formation of this cyclic nucleotide, and labeled more strongly with [^{14}C]adenine than the total cell pool of ATP. Papaverine doubled the cAMP content in the thymocytes and increased its specific radioactivity by 21%. Under the influence of papaverine, which is a powerful inhibitor of cAMP-dependent phosphodiesterase [13], accumulation of cAMP in the thymocytes continued on account of the same ATP compartment with high specific radioactivity as in the control experiment, when the cells were incubated in the absence of the effector. In this situation the small but significant rise in specific radioactivity can be explained by a decrease in the total intracellular cAMP content of its pool, which is connected with regulatory subunits of protein kinases [5], and, being metabolically inert, is poorly labeled with [^{14}C]adenine.

Papaverine not only increased the concentration and specific radioactivity of cAMP in the thymocytes, but also considerably intensified ATP catabolism, which in turn was accompanied by a considerable decrease in specific radioactivity of ATP. This decrease indicates that catabolism of ATP under the influence of papaverine also takes place on account of the compartment preferentially labeled with [^{14}C]adenine compared with the total cell nucleotide triphosphate pool, but according to calculations based on data in Table 1, its specific radioactivity [21.2 ± 1.5 cpm/pmole) was less than that of the ATP compartment serving as the source of cAMP.

Adenosine, added to thymocytes, like papaverine, almost doubled the cAMP content but, unlike papaverine, it reduced the specific radioactivity by 36% relative to the control. This decrease took place because adenosine, on conversion into ATP, increases its content by 1.7 times, with a corresponding dilution (by 40%) of the label and a fall in the specific

radioactivity of ATP. Meanwhile, the use of labeled [^{14}C]adenosine under similar experimental conditions showed that 1.53 ± 0.42 pmoles cAMP/10^7 cells (n = 5), or about 45% of the total cyclic nucleotide, was formed from it. Comparison of these data indicate that the increase in cAMP content in rat thymocytes under the influence of adenosine took place almost entirely on account of its direct utilization by phosphorylation for ATP synthesis.

In combination with papaverine, adenosine raised the cAMP level almost sixfold, which was more than the expected combined effect. The specific radioactivity became considerably lower or showed a marked tendency to fall compared with that observed under the influence of papaverine or adenosine alone. Less cAMP was formed from [^{14}C]adenosine in the presence of papaverine and under identical experimental conditions: only 0.52 ± 0.05 pmole/10^7 cells (n = 5) or about 6% of the total cyclic nucleotide. Under these circumstances the character of the changes observed in the level and specific radioactivity of cAMP cannot be explained by its formation from adenosine. Alternative pathways by which adenosine could raise the cAMP level in lymphocytes are activation of adenylate cyclase from outside, through the approximate plasma membrane receptors [8], and inhibition of cAMP-dependent phosphodiesterase [4]. This last mechanism seems improbable, considering that adenosine does not accumulate in cells. Consequently, adenosine, in the presence of papaverine, raises the cAMP level through activation of adenylate cyclase, the substrate for which is ATP with specific radioactivity similar to that of the ATP degrading under the influence of papaverine. Hence papaverine can perhaps catabolize ATP from the same compartment as that which supplies the adenylate cyclase reaction that is sensitive to adenosine.

AMP (alone or together with papaverine) acts in the same way as adenosine on the content and specific radioactivity of cAMP (Table 1). The reason is that it is converted by means of ecto-5'-nucleotidase into adenosine. Different ATP compartments, destined for cAMP formation and unequally labeled with [^{14}C]adenine, also were found during a study of the action of neuromediators on brain tissue slices [10, 11].

The results thus indicate that adenosine or AMP raise the level of cAMP, of which they are its precursors, in thymocytes, but in the presence of papaverine, they exert their action from outside, through adenylate cyclase. Two ATP compartments, preferentially labeled by [^{14}C]adenine compared with the total cell ATP content, and destined for cAMP formation, were found in rat thymocytes. cAMP is formed from the compartment with higher specific radioactivity in thymocytes incubated in the absence of effectors or on the addition of papaverine. The other compartment, with lower specific radioactivity, serves as the source of ATP for adenosine- or AMP-sensitive adenylate cyclase, and it catabolizes under the influence of papaverine.

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