applied here, but in absence of oxime, was determined to be $3 \times 10^{-3}\,min^{-1}$ [10]. The kinetical analysis using this value and the measured time course of reactivation, however, did not lead to satisfactory results. This irregular behaviour was likewise found by DeJong and Wolring and its possible reasons are exhaustively discussed in their paper [9]. Nevertheless, the percentage figures in Table 1 allow a rough estimation: with HS 6 or HGG 12, for instance, reactivation and aging appear to proceed with similar rates, whereas with HJ 6 the reactivation rate constant should be more than 10 times higher.

Group 1 compounds comprises three pairs of isomers with respect to the location of the substituent in the non-oxime ring ('ring 2'). *In vitro*, reactivation is optimal with the substituent located in the 4-position. For the compounds HS 6 and HJ 6 DeJong and Wolring [9] reported analogous results. Accordingly, we found throughout in previous investigations, that bovine red cell AChE, when inhibited by paraoxon, tabun and sarin [15, 16], prefers the substitution in position 4 of this 'second' pyridine ring. The same holds for the respective ED_{50} data from mice [16], except soman; against soman the 3-substituted (with respect to the 'ring 2') bispyridinium-2-aldoximes in general lead to lower ED_{50} values than the 4-isomers [5, 17, 18]. The same gradation for HGG 12/HGG 9 was found by Maksimovic *et al.* [19] using mice and by Weger and Szinicz with beagles for HS 6/HJ 6 [20]. Kepner and Wolthuis [21], however, found HJ 6 to be superior to HS 6 in rats; Clement and Lockwood [22] came to the same result with mice. Their experimental procedures are not so different from those used here, and by the other authors, that this fact alone could explain the differing results. In view of a possible extrapolation to the behaviour of the oximes in man this discrepancy needs further investigation.

The second group of oximes represents a series of homologous compounds differing only in the number of methylene groups in the side chain of the 'second' pyridine ring. The ED_{50} values reach a minimum with the hexyl compound, whereas the maximal reactivating potency *in vitro* was found with the octyl-homologue.

In a previous paper [18], we reported the protective effects of homologous bis-(4-tert-butylpyridinium)alkylene salts against soman in mice and towards bovine red cell AChE. There we found optimal protection *in vitro* with the five-membered, and *in vivo* with the hexylene chain.

The reaction mechanisms *in vitro* are obviously different for the homologous oximes and the oxime-free salts, one being reactivation and the other protection of the active site. From the *in vivo* results, however, regarding the discrepancy to the reactivation studies and the remarkable coincidence in favouring a six-membered alkyl chain, no reliable conclusion can be drawn about the mode of action of the oximes.

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β-Adrenoreceptors display different efficiency on lymphocyte subpopulations

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Catecholamines modulate cellular activity via specific receptors on the cell surface. Such receptors were first identified on cell membrane preparations [1] and more recently the availability of ligands of high affinity and high specific activity has made possible their quantitation on intact cells including lymphocytes [2–5]. The binding of a β-agonist to its receptor triggers an increase in adenylate cyclase activity with a resultant increase in the synthesis of

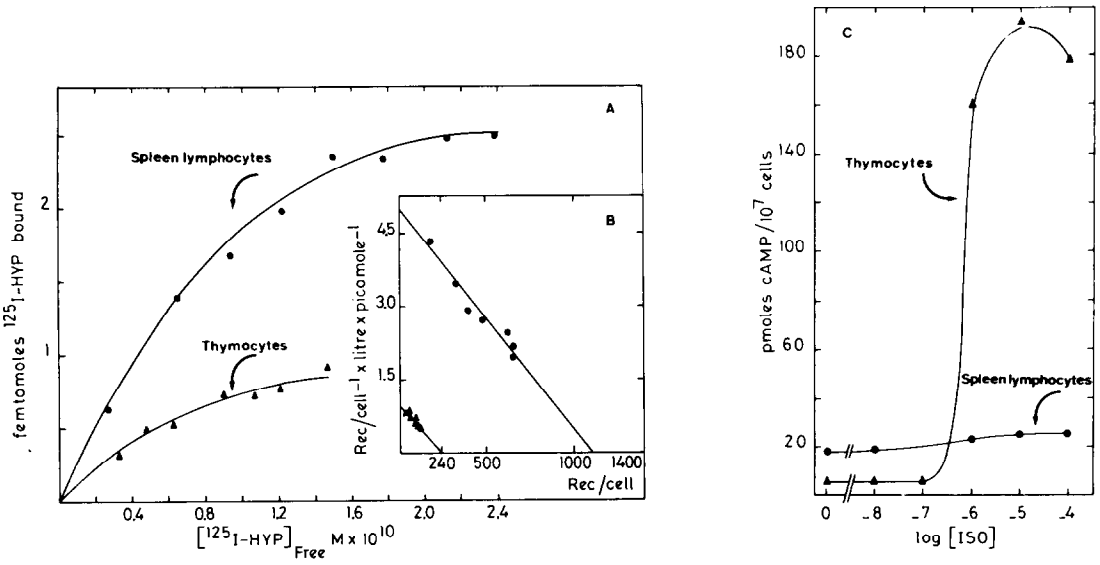

Fig. 1. Young (20–40 days) female Swiss mice were used as source of lymphocytes. Single cell suspensions were obtained from the thymus and from the spleen by teasing the tissues in ice-cold serum free culture medium (RPMI 1640, Flow Lab.). The spleen cells were treated with amonium chloride to remove red blood cells (this treatment did not change the binding capacity nor the lymphocyte response to cAMP stimulants). The cell yield was approximately 10^8 cells per thymus and 6×10^7 cells per spleen. For binding studies, lymphocytes (5×10^6 thymus cells or 2×10^6 splenic cells) were incubated at 37° in 0.5 ml of PBS (phosphate buffered saline) containing 0.9 mM $CaCl_2$ and 0.5 mM $MgCl_2$ with different amounts of ^{125}I-HYP and 10^{-5} M phentolamine. After 60 min, 5 ml of 50 mM Tris–HCl pH 7.4, 10 mM $MgCl_2$ were added to the incubated cells. The suspension was immediately filtered on a GF/C Whatman glass fiber filter. The filter was washed with 25 ml of the same buffer at 37°, and counted in a Packard gamma counter. These methods have been previously reported in detail [2]. (A) ^{125}I-HYP specifically bound to 5×10^6 thymus cells (△) and to 1×10^6 splenic cells (○) as a function of the concentration of free ^{125}I-HYP. Specific binding was obtained by subtracting the ^{125}I-HYP bound in the presence of 10^{-7} M (−)-propranolol from total binding. 10^{-7} M (−)-propranolol specifically displaces the ^{125}I-HYP bound to sites having a high affinity for the (−) configuration of the ligand. (B) Scatchard plot calculated from (A) gives saturation values of 1.150 β-receptors per spleen lymphocyte and 240 β-receptors per thymocyte with a K_D value of 2.2×10^{-10} M for both populations of cells. In seven consecutive experiments, K_D values for both thymus and spleen cells were comprised between 1.3 to 2.2×10^{-10} M; whereas the ratio between the number of receptors on spleen versus thymus lymphocytes was always comprised between 3 and 5. (C) The effect of different concentrations (−)-isoproterenol on cAMP content of spleen lymphocytes (○) and thymocytes (△). Total cAMP (intracellular and secreted) was determined according to Brooker *et al.* [31]. Cells (5×10^6) were incubated for 5 min at 37° in 1 ml incubation medium with or without isoproterenol. The reaction was stopped by adding TCA (6%, final concentration) to the incubation medium. After centrifugation and extraction of the TCA with ether, the supernatant was acetylated. Acetylated cAMP was measured by RIA using ^{125}T-methyltyrosine ester cAMP. The antibody against cAMP was kindly provided by Dr. K. Ishizaka (Baltimore). Cell survival after incubation was greater than 85 per cent as measured by eosin exclusion. Mean values ± S.D. of 13 other consecutive assays were as follows: (1) basal cAMP: 3.76 ± 3.5 pM/10^6 spleen cells vs 2.5 ± 2.2 pM/10^6 thymic cells ($P < 0.05$; paired *t*-test); (2) stimulation index (ratio between cAMP content of stimulated and unstimulated cells) to isoproterenol (10^{-4}, 10^{-5} M): 2.67 ± 0.5 in spleen cells vs 6.40 ± 1.7 in thymocytes ($P < 0.001$) (3) stimulation index to PGE_1 (10^{-5}, 10^{-6} M): 3.1 ± 0.6 for splenocytes vs 11.5 ± 2.9 for thymocytes ($P < 0.001$) (7 experiments) (4) stimulation index to cholera toxin (0.5 μg/ml): 8.8 ± 3.1 for spleen cells vs 35.6 ± 19.7 for thymocytes ($P < 0.001$) (8 experiments).

3′,5′- adenosine monophosphate (cAMP). The measurement of changes in intracellular levels of cAMP or in adenylate cyclase activity is currently used to estimate cellular sensitivity to β-agonists. Theoretically, these two types of measurements should give results proportional to receptor density, lymphocytes having more receptors demonstrating increased responsiveness to β-agonists. Although this concept has been experimentally demonstrated in some systems [6–8] it cannot be generalized as clearly illustrated by the observations of Haga *et al.* [9] who were able to select a mutant cell which possessed β-receptors as well as adenylate cyclase but was unable to respond to a β-agonist. It is now well established that stimulation of adenylate cyclase by hormones, including catecholamines, involves at least three physically separable components: the hormone receptor, the catalytic adenylate cyclase and a regulatory subunit termed N [10]. N is a receptor–cyclase coupling protein required for stimulation of adenylate cyclase by the hormone–receptor complex. Recent data further indicate that the coupling of receptor to cyclase is also conditioned by the fluidity and the lateral mobility of cell membrane [11–14]. In a previous study we have demonstrated a difference in the number of β-receptors present on T and B lymphocytes from human tonsils [2]. (T lymphocytes demonstrated 200 receptors per cell whereas B lymphocytes demonstrated 600 per cell. The

Table 1.

Cells tested		cAMP (pM/10^6 cells) Basal	Iso	PGE_1	Cholera toxin	R_T‡	R_T§	K_D‖
A Thymocytes	(88 ± 10)*	4.3 ± 0.5†	50 ± 3.5	70 ± 12	342 ± 53	220 ± 49.5	1.04 ± 0.23	1.6 ± 0.4
Spleen cells	(40 ± 7.2)	9.8 ± 1.1	12.6 ± 1.5	18.5 ± 2.7	63.3 ± 25	725 ± 164	3.44 ± 0.78	1.4 ± 0.2
Spleen T cells	(86 ± 6.5)	8.8 ± 1.9	14.4 ± 4.4	20 ± 3.4	72 ± 24	509 ± 42.3	2.41 ± 0.20	1.8 ± 1.3
Spleen B cells	(8 ± 4.5)	9.3 ± 3.3	12.5 ± 4	14.4 ± 4.4	69 ± 14	693 ± 73.9	3.28 ± 0.35	1.1 ± 0.4
B Thymocytes unfractionated		6.7 ± 1.3¶	56.4 ± 4.1	78.5 ± 10				
Low density thymocytes		12.9 ± 1.8	47.4 ± 6.2	51.4 ± 7		**0.83 ± 0.15	fM/10^7 cells	
High density thymocytes		6.9 ± 1.4	59.2 ± 3.7	98.5 ± 13		0.52 ± 0.12	fM/10^7 cells	

* Percentage of theta positive cells.
† Mean ± S.D. of three consecutive experiments.
‡ R_T: number of receptors per cell.
§ Receptor number/μm^2 ($r = 4.1\ \mu$).
‖ K_D: dissociation constant × 10^{-10} M.
¶ Mean ± S.D. of four experiments, the difference between high and low density fractions is statistically significant ($P < 0.01$; covariance analysis).
** Specific binding after incubation in the presence of 0.1×10^{-10} M ^{125}I-HYP, the difference between low and high density thymocytes is highly significant (paired t-test, $P < 0.01$).

Suspension of thymocytes and spleen cells, prepared as described in legend to Fig. 1, were stored in ice-cold incubation medium until assayed. Splenic T lymphocytes were obtained by filtration through nylon wool column [29]; splenic B lymphocytes were prepared by incubation with rabbit antiserum against mouse brain-associated theta-antigen and guinea pig complement [30]. Anti-theta serum was a gift of Dr. K. Ishizaka (John Hopkins University, Baltimore, U.S.A.). The viability of the cells was higher than 90% as measured by eosin exclusion test. The lymphocyte preparations were evaluated for the percentage of theta positive cells by a cytotoxicity test with eosin and anti-theta serum in the presence of complement. Thymocytes were fractionated by centrifugation on a discontinuous bovine serum albumin (BSA) density gradient that consisted of layers 21/24/27/30% BSA (Poviet Lab). The recovered gradient-fractionated cells were divided into two populations by pooling cells sedimenting in the top two interfaces (low density thymocytes) and in the botton two fractions (high density thymocytes). This was done to obtain number of cells sufficient for the experiments; 67% of thymocytes originally placed on the gradient were recovered with 96% of them obtained in the high density fraction. Heavy thymocytes are of cortical origin and are more immature than the medullary light thymocytes [22, 23]. These different preparations of lymphocytes were assayed simultaneously for cAMP synthesis and for specific ^{125}I-binding. cAMP synthesis was measured after 15 min incubation at 37° in the presence or absence of isoproterenol (10^{-5} M, Sigma) or prostaglandin E_1 (10^{-5} M, Sigma) and after 60 min incubation in the presence or absence of cholera toxin (0.5 μg/ml; Sigma Lab.). The basal cAMP was the same after 15 and 60 min incubation at 37°. Owing to the low cellular recovery, ^{125}I-HYP binding to thymocytes fractions was performed with only one concentration of the ligand (0.1×10^{-10} M).

affinity of the ligand ^{125}I-HYP was the same in the two subpopulations; $K_D = 2 \times 10^{-10}$ M). These results were in apparent contrast with some previous reports showing greater isoproterenol stimulation of human T vs B lymphocytes isolated from blood, tonsil or adenoids [15, 16]. We found the same discrepancy between our initial observations of the number of β-receptors on murine thymus and spleen cells and the published data on the respective sensitivity of these cells to β-agonists [17–20]. In the present study, this paradox was investigated by measuring on the same cell preparations the density of β-receptors and the response generated by isopreterenol and other cAMP stimulants. The results demonstrate an inverse relationship between the number of β-receptors per cell and cAMP response when studied on intact lymphocytes isolated from murine thymus or spleen. The data further suggest that during the maturation of T lymphocytes there is a progressive loss of receptor–cyclase coupling.

The stages through which T lymphocytes pass are the following: Pre T lymphocytes migrates from the bone marrow to the cortex of the thymus, then pass eventually to the medulla before entering into the circulation where they colonize the secondary lymphoid organs (spleen, lymph nodes). These are named T mature or T peripheral lymphocytes. In the thymus, the thymocytes pass through different development stages. A density gradient centrifugation of the thymocytes has been used to separate the more differentiated thymocytes from the immature thymocytes [21, 22].

Figure 1 compares the number and affinity of β-adrenergic receptors with the synthesis of 3′,5′-cAMP in response to isopreterenol in thymus and spleen lymphocytes. In this experiment, thymocytes have 4.6 times fewer receptors per cell than spleen lymphocytes; in contrast, their response to isopreterenol is 20 times higher. Note that the affinity of the receptors for the ligand is the same in the two lymphocyte populations. Additional experiments (not shown) showed that this discrepancy cannot be explained by a different kinetic of cAMP synthesis in the two lymphocyte populations and still persists after phosphodiesterase inhibition by isobutylmethylxanthine (1 mM).

The observation of a greater responsiveness of thymus lymphocytes bearing fewer β-adrenergic receptors than spleen lymphocytes cannot be accounted for by the heterogeneity of the lymphocyte populations used in our experiments. This has been demonstrated by comparing the data obtained: (1) on high- and low-density thymocytes versus unfractionated thymus cells; (2) on splenic lymphocytes enriched in T or B cells vs unfractionated spleen and thymus lymphocytes (Table 1). Thymocytes were fractionated by density gradient centrifugation, and separate into immature cells of high density and more differentiated thymocytes with lower density. Our experiments show that immature thymocytes have a lower basal level of cAMP, a higher response to isoproterenol but a lower specific ^{125}I-HYP binding in comparison to the more differentiated low-density thymocytes. Comparision of spleen cell preparations enriched in T or B lymphocytes to unfactionated spleen and thymus lymphocytes indicates that thymocytes are more responsive to isoproterenol than peripheral T cells ($P < 0.01$; paired t-test) whose response is similar to that of "purified" B cells; conversely, the number of β-receptors per cell is higher in B than in peripheral T cells ($P < 0.05$) who display more receptors than thymocytes ($P < 0.01$). This is also true if the results are expressed in receptor per membrane surface unit. Indeed the diameter of the different lymphocytes population tested are all within the range 8–9 μm. Our finding of a decline in cellular sensitivity to β-agonists during maturation of T lymphocytes (starting from the high density, immature, non competent cortical thymocyte to the fully differentiated and immunocompetent spleen T cell) is in good agreement with previous reports [17–20] but we further show that this loss of responsiveness to β-stimulants is not accompanied by a parallel decrease but rather by an increase in the number of β-receptors. Further analysis of our data (Table 1) shows a parallelism between the response to isoproterenol of a given lymphocyte population and its response to PGE_1 which stimulates cAMP synthesis via specific receptors distinct from β-receptors. This suggests that the responsiveness of a lymphocyte subset is conditioned by a structure located distal to the receptor.

The activiation of hormone-sensitive adenylate cyclase requires the participation of a regulatory protein (N) located in the plasma membrane. This receptor coupling unit binds guanine nucleotides [23] and has a GTPase activity [24]. Cholera toxin irreversibly binds to N and inhibits its GTPase activity, thereby allowing the endogenous GTP to stimulate permanently the adenylate cyclase [25]. Our additional observations that cholera toxin is a more potent stimulant of cAMP synthesis in thymus than in spleen cells (unfractionated and T or B enriched fractions) indicate that these populations differ either by their content (or activity) in catalytic adenylate cyclase either or by the linkage of the regulatory N protein with the receptors. This last possibility is in agreement with the recent observations that the decline in catecholamine responsiveness in maturing rat erythrocytes is accompanied by a loss in the interactions between β-adrenergic receptors and N regulatory protein [26]. The results are also in keeping with recent findings of an increase of β-receptor density in differentiating muscle [27] and during erythroid differentiation of Friend leukemic cells [28].

In summary, we show here that the responsiveness to β-adrenergic stimulants of lymphocyte subpopulations differing by their origin and/or maturation is not determined by the number of β-receptors present on the cell surface but possibly by the molecule(s) coupling the β-receptor to adenylate cyclase or by the linkage of the coupling protein with the receptor. This account for the present observation on maturing T lymphocytes of a progressive decline in responsiveness to β-stimulants contrasting with a parallel increase in the number of β-receptors. This cannot be explained by (1) the kinetic of the response to isopreterenol; (2) the activity of cAMP dependent phosphodiesterase; (3) the affinity of the β-receptors.

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Phenolsulphotransferase in human placenta

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Sulphate conjugation is an important pathway in the metabolism and excretion of catecholamines and many other phenolic compounds [1]. This reaction is catalysed by the cytoplasmic enzyme, phenolsulphotransferase (PST) which transfers the sulphate group from 3′-phosphoadenosine 5′-phosphosulphate (PAPS) to a phenolic acceptor [2]. PST is widely distributed in human tissues. It is highly active in the intestine, present in platelets and activity has also been noted in the placenta [3]. Placental PST may be important for the inactivation of endogenous phenols, dietary phenols or phenolic drugs present in the maternal circulation and prevent their access to the fetus.

PST in human platelets, brain and jejunum exist in two functional forms which we have denominated M and P forms [4]. PST M acts specifically on monoamines and related compounds, e.g. dopamine, tyramine, noradrenaline, adrenaline [5], α-methylnoradrenaline [6] and their metabolites such as 4-hydroxy-3-methoxyphenylglycol (HMPG) [4]; PST P acts on low concentrations of phenol [4]. Salicylamide, in low concentration, is also a specific P substrate although both it and phenol become M substrates at higher concentration [5, 7] (Bonham Carter *et al.*, submitted for publication). These two forms can be distinguished by their thermostability, the M form being more thermolabile, and by the action of the inhibitor, dichloronitrophenol (DCNP) which selectively inhibits the P form [4]. The two forms are controlled independently in different individuals [8]. Because of the possible functional importance of the placental enzyme, the aim of the present study was to study its activity towards a wide range of phenolic compounds and examine its sensitivity to DCNP, in order to determine whether both forms of the enzyme operate at this site and to measure their relative activities.

Materials and methods

A solution of 3′-phosphoadenosine 5′-phospho[^{35}S]sulphate (PAPS), 4.2 Ci/mmol, in 50% ethanol was purchased from New England Nuclear, Boston, Mass, USA and stored at −20°. Unlabelled PAPS was obtained from PL Biochemicals, Inc., Milwaukee, WI, USA. The substrates were purchased from Sigma Chemical Company, Poole, Dorset, U.K. and used without further purification. DCNP (2,6-dichloronitrophenol) was purchased from Fluka AG, Switzerland and also used without further purification.

Small segments from four freshly delivered human placentae were cut into smaller portions and as much blood as possible expelled from them. Homogenates (10% w/v) of each in 10 mM phosphate buffer, pH 7.4, were pooled and centrifuged at 100,000 *g* for 1 hr. The supernatant was stored in small aliquots at −20° in polypropylene test tubes.

The enzyme assay used was that described by Rein *et al.* [9] based on the method of Foldes and Meek [10]. The reaction mixture contained 100 μl 10 mM phosphate buffer, pH 7.4, 30 μM phenolic substrate (unless otherwise stated), 0.6 μM [^{35}S] PAPS and 10 μl placental supernatant. Inhibitor studies were carried out as described by Rein *et al.* [4]. A range of DCNP concentrations, from 10^{-4} to