The amount of bound $^3$H-estradiol in the nuclear, microsomal and cytosol fractions from rat uteri, was well as those from human blood leukocyte preparations, is much lower than that from the 24,000 *g* fractions. Non-radioactive estradiol does not decrease the amount of bound radioactivity in the nuclear, microsomal and cytosol fractions. Hydrogen peroxide does not increase the amount of bound radioactivity in those fractions, as was observed in the 24,000 *g* fractions. The guiacol reaction demonstrates only the presence of peroxidase in the uterine and blood leukocyte 24,000 *g* fraction, but not in the nuclear, microsomal and cytosol fractions.

The Figure shows the results expressed as SCATCHARD plots. The estrogen receptors from both the rat uterine 24,000 *g* fraction and the eosinophil-rich human blood leukocyte 24,000 *g* fraction have a constant of dissociation of $5.6 \times 10^{-10}$ *M*. The total number of binding sites per eosinophil (from the blood leukocyte preparations) is 7,400 sites per cell. In both the rat uterine and the eosinophil-rich human blood leukocyte 24,000 *g* fractions, there is no detectable specific estrogen binding in the presence of non-radioactive estradiol-17$\beta$.

*Discussion.* The present report describes high affinity and low capacity estrogen receptors in the rat uterine and the human eosinophil-rich blood leukocyte 24,000 *g* fractions. No estrogen receptors were detected in the cytosol, microsomal and nuclear fractions from rat uteri or from human blood leukocyte preparations, probably because of the long incubation at 37 °C, which has been reported to destroy the uterine cytosol receptors[7].

The estrogen receptors from the blood leukocyte 24,000 *g* fraction were only clearly demonstrated when the blood was obtained from patients with a high count of blood eosinophils, suggesting that these estrogen receptors are only present in the eosinophil leukocytes.

The estrogen receptors from the 24,000 *g* fraction of rat uterus and those from the 24,000 *g* fraction of eosinophil-rich blood leukocyte preparations have three characteristics in common: They have a similar constant of dissociation, they increase the binding of $^3$H-estradiol in the presence of a low concentration of hydrogen peroxide and they are found in the 24,000 *g* fraction. These characteristics suggest that the 24,000 *g* fraction eosinophil-rich blood leukocyte receptors are identical with the uterine estrogen receptors found in the 24,000 *g* fraction.

An increase in estrogen binding by the blood leukocyte and the uterine 24,000 *g* fractions occurs in the presence of a low concentration of hydrogen peroxide. Similarly, an increase in estrogen binding and retention by the uterine eosinophils in the presence of a low concentration of hydrogen peroxide has been reported in radioautographic studies[15,16], suggesting that the estrogen receptors found in the 24,000 *g* fractions correspond to the estrogen receptors previously described in uterine eosinophils[2-6,8-11,15-17]. Furthermore, electron microscope radioautography demonstrated the localization of the estrogen eosinophil receptors in the 'specific lysosomes' of the eosinophil leukocytes[17], known as 'peroxidasosomes'[18], which sediment in the 24,000 *g* fraction[19].

Experimental data has led us to conclude that the estrogen receptors described in the blood leukocyte and the uterine 24,000 *g* fractions correspond to the uterine eosinophil estrogen receptors previously found in uterine radioautograms, confirming their existence in the uterus, in addition to the cytosol-nuclear receptor system.

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## The Desulphation of Hexosamine Sulphates by Arylsulphatase B[1]

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*Summary.* The sulphation of Carbobenzoxyglucosamine by chlorosulphonic acid resulted in formation of *N*-carbobenzoxyglucosamine-4,6-disulphate. UDP-galactosamine 4-sulphate and glucosamine 4,6-disulphate were the competitive inhibitors of arylsulphatase B. Arylsulphatase B can hydrolyze UDP-galactosamine 4-sulphate and glucosamine 4,6-disulphate but not galactosamine 6-sulphate.

Lysosomal arylsulphatase B has been extensively purified from various mammalian tissues[2-4]. It can be clearly distinguished from the corresponding arylsulphatase A both by its lower molecular weight and higher isoelectric point[2]. Although the physiological role of arylsulphatase A has been well established[5-7], the role of arylsulphatase B is still obscure. However, it has been suggested[8] that the latter may have a role in mucopolysaccharide metabolism. Further, it has recently been reported that arylsulphatase B activity is markedly diminished in the tissues of patients with Maroteaux-Lamy syndrome, which is characterized by the abnormal excretion of dermatan sulphate[9,10]. In a preliminary report STEVENS et al.[11] have reported that a partially purified preparation of human placenta arylsulphatase B could desulphate UDP-N-acetylgalactosamine 4-sulphate.

In the present work, attempts were made to prepare hexosamine sulphates for using them as substrates for arylsulphatase B. The hexosamine sulphates were also coupled with CH-sepharose in an attempt to make an affinity chromatography column for arylsulphatase B.

*Methods.* The homogeneous preparation of arylsulphatase B was prepared by the method of FAROOQUI and ROY[4], and assayed by the method of ALLEN and ROY[2] using nitrocatechol sulphate as a substrate. The purified enzyme preparation had a specific activity of 130 µmole/min/mg.

*Preparation of hexosamine sulphates.* Carbobenzoxyhexosamines were prepared by the method of CHARGAFF and BOVARNICK[12]. They were crystallized 3 times with 30% methanol before sulphation. The sulphation of carbobenzoxyhexosamines was done by the method of ADAM[13]

Table I. Effect of hexosamine sulphates on arylsulphatase B activity

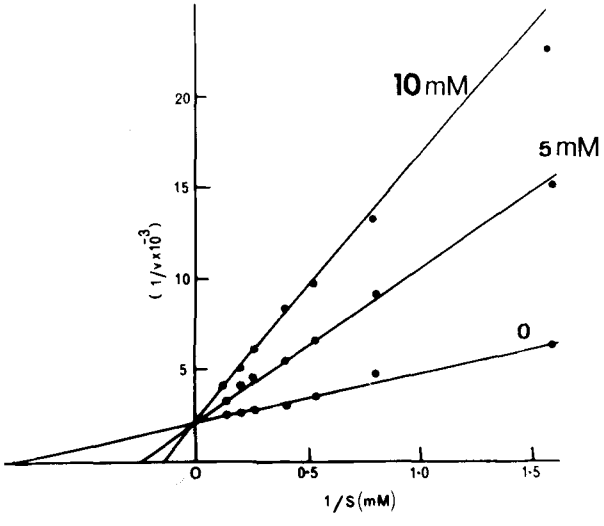
| Hexosamine sulphate | Concentration (m*M*) | Inhibition (%) |
|---|---|---|
| UDP *N*-acetylgalactosamine 4-sulphate | 5 | 40 |
| | 10 | 60 |
| Carbobenzoxyglucosamine 4,6-disulphate | 5 | 18 |
| | 10 | 50 |
| Glucosamine 4,6-disulphate | 5 | 20 |
| | 10 | 52 |
| Carbobenzoxygalactosamine 6-sulphate | 5 | 0 |
| | 10 | 0 |
| Galactosamine 6-sulphate | 5 | 0 |
| | 10 | 0 |

The enzyme was assayed by the method of ALLEN and ROY[2] using *p*-nitrocatechol sulphate as substrate. 10 µg enzyme protein was used.

Table II. Rate of hydrolysis of various hexosamine sulphates by ox liver arylsulphatase B

| Substrate | Sulphate formed (nmol/min/mg protein) |
|---|---|
| UDP *N*-acetylgalactosamine 4-sulphate | 95 |
| Carbobenzoxyglucosamine 4,6-disulphate | 26 |
| Carbobenzoxygalactosamine 6-sulphate | 0 |
| Glucosamine 4,6-disulphate | 35 |
| Galactosamine 6-sulphate | 0 |

The enzyme was assayed as described in the methods.




Effect of carbobenzoxyglucosamine 4,6-disulphate on the activity of ox liver arylsulphatase B. The concentration of carbobenzoxyglucosamine 4,6-disulphate is indicated and the enzyme assayed by the method of ALLEN and ROY[2].

using chlorosulphonic acid, and the carbobenzoxyhexosamine sulphates were isolated as barium salts from the lower layer of sulphation mixture by the method of LLOYD[14]. The crude barium salts of carbobenzoxyhexosamine sulphate, after 3 crystallization with water, were converted to potassium salts by passage through a column of Dowex 50 × 4 $K^+$ form. The purified $K^+$ salts of carbobenzoxyhexosamine sulphates were obtained after 3 precipitations with cold absolute ethanol by the method of LLOYD[14].

The decarbobenzoxylation of carbobenzoxyhexosamine sulphate was done by the method of ADAM[13], and hexosamine sulphates were crystallized 3 times before use. UDP-*N*-acetylgalactosamine 4-sulphate was prepared by the method of DONOVAN et al.[15]. A 10 m*M* solution of various hexosamine sulphates in 0.5 *M* sodium acetate buffer, pH 5.0 was incubated for 1 h at 37 °C with 50 µg of enzyme protein and the liberated sulphate was determined by the method of DODGSON[16].

*Results and discussion.* As reported by ADAM[13], the sulphation of carbobenzoxygalactosamine resulted in formation of carbobenzoxygalactosamine 6-sulphate ($C_{14}H_{18}O_{10}$ NSK; Calculated: C, 38.98; H, 4.17; S, 7.42 and K, 9.04; Found C, 39.00; H, 4.0; S, 7.4 and K, 9.0). The sulphation of carbobenzoxyglucosamine under similar conditions gave carbobenzoxyglucosamine disulphate ($C_{14}H_{17}O_{13}NS_2K_2 \cdot 2H_2O$, Calculated: C, 28.71; H, 3.58; N, 2.39; S, 10.94 and K, 13.33; Found: C, 28.41; H, 3.31; N, 2.17; S, 10.09 and K, 13.00).

*Identification of carbobenzoxyglucosamine disulphate.* The sulphate group in carbobenzoxyglucosamine disulphate could be esterified either 3,6 or 4,6 carbon position of glucosamine. It may also be possible that the present compound is a mixture of both 3,6 or 4,6-disulphates. The evidence to support the view that sulphate groups are localized at 4 and 6 carbon of *N*-carbobenzoxyglucosamine was obtained by periodate oxidation. The present compound did not consume periodate under the conditions used by various workers[17–19]. Further it neither

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produced formaldehyde nor glycolaldehyde on periodate oxidation. If sulphate groups had been esterified at 3,6 or 3,4 carbon of *N*-carbobenzoxyglucosamine, the compound would have consumed periodate, with the production of formaldehyde in the case of 3,4-disulphate, and glycolaldehyde sulphate in the case of 3,6-disulphate[17–19]. Thus, in the present compound, the sulphate groups are esterified at 4 and 6 carbon of *N*-carbobenzoxyglucosamine.

The effect of various hexosamine sulphates on arylsulphatase B activity is shown in Table I. At 10 m*M* carbobenzoxygalactosamine 6-sulphate or galactosamine 6-sulphate has no effect on enzyme activity, whereas UDP-*N*-acetylgalactosamine 4-sulphate at 10 m*M* produces 60% inhibition of arylsulphatase B activity. Both carbobenzoxyglucosamine 4,6-disulphate and glucosamine 4,6-disulphate inhibited the enzyme activity of 50% at 10 m*M*. The nature of inhibition in all cases was a competitive one and for carbobenzoxyglucosamine 4,6-disulphate is shown in the Figure. The glucosamine 4,6-disulphate was also coupled with CH-sepharose by the method of ALLEN and NEUBERGER[20] in an attempt to make an affinity chromatography column for arylsulphatase B. The enzyme did not show any affinity for glucosamine 4,6-disulphate bound CH-sepharose under variety of conditions of pH and ionic strength.

The rate of hydrolysis of various hexosamine sulphates by arylsulphatase B is shown in Table II. There was no release of sulphate either from carbobenzoxygalactosamine 6-sulphate or galactosamine 6-sulphate. The release of sulphate was approximately 2.5 times higher in the case of UDP-*N*-acetylgalactosamine 4-sulphate compared with glucosamine 4,6-disulphate. Here it must be recalled that earlier attempts to show the presence of sulphatase acting on hexosamine sulphate have been unsucessful[21], probably because the substrates used were hexosamine 6-sulphates. The present study confirms the previous reports by showing that arylsulphatase B has no activity towards galactosamine 6-sulphate. Further it lends additional support to the suggestions[11,22,23] that the *N*-acetylgalactosamine 4-sulphate in dermatan sulphate and chondroitin 4-sulphate is a naturally occurring substrate for arylsulphatase B. The hydrolysis of glucosamine 4,6-disulphate by arylsulphatase B also indicates that the enzyme has a high specificity for the position of sulphate moiety and the nature of hexosamine moiety is not so important.

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## Close Correlation between Levels of Cholesterol and Free Fatty Acids in Lymphoid Cells

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*Summary.* A close correlation was found between the levels of free cholesterol and free fatty acids in lymphoid cells from thymus, spleen or lymph node of mice and guinea-pigs. This relationship suggests a possible role of cholesterol regulating the fatty acid levels in lymphoid cells.

It is well known that the structural lipid of mammalian cell membrane consists primarily of phospholipid and cholesterol, and their proportions and structure are very important to the properties and functions of cell membrane[1–4]. Free cholesterol has recently been shown to be involved in the membrane fluidity relating to the rigidity of surface membrane of lymphocytes[5]. Studies on lymphocyte lipids have revealed that the cholesterol levels in normal lymphocytes from man and animals differ markedly from that in leukemic cells[5–7]. In a previous communication we reported that growth of Ehrlich's ascitic carcinoma in mice results in increase of free cholesterol and free fatty acids in lymphoid cells from thymus, spleen and cervical lymph node, but decrease of these lipids in the cells from mesenteric lymph node[8]. The present study demonstrates a close correlation between the levels of free cholesterol and free fatty acids in lymphoid cells from thymus, spleen or lymph nodes of mice and guinea-pigs.

The thymus, spleen and lymph nodes (cervical and mesenteric lymph node) were obtained from the following groups of female mice (*ddN* strain): mice bearing with Ehrlich's ascitic carcinoma, mice bearing with solid tumors of Ehrlich's cells on tail and animals without tumors (normal mice, 24–26 g). These 3 groups of mice were fed with diet and given water ad libitum before the experiments. 1 group of mice receiving the i.p. inoculation of tumor cells ($5 \times 10^6$ cells/mouse) were killed by cervical dislocation on the 5th and 10th day after inoculation (mice bearing with ascitic carcinoma)[8]. Another group of mice implanted s.c. with tumor cells midway up the tail ($4 \times 10^6$ cells/mouse) was killed 7, 14 and 21 days after implantation, except the animals which showed no growth of solid tumors (mice bearing with solid tumors)[9]. The mouse tissues were also obtained from normal animals deprived of diet for 48 h before the experiments. Preparing of lymphoid cells from mouse tissues and lipid quantitation of the cells were performed by the method described previously[8,10]. In addition, lymphoid cells were prepared from spleen and inguinal lymph node of guinea-pigs (Hartley strain, 500–700 g) fed with diet ad libitum before the experiments[10]. Before lipid quantitation, the guinea-pig lymphoid cells were suspended in Krebs-Ringer phosphate buffer (pH 7.4)[11] containing 2% of bovine albumin fraction V (Armour Laboratories) and incubated at 37 °C for 2 h in the presence of adrenergic agents (isoproterenol, epinephrine or norepinephrine) at concentrations of $10^{-5}$–$10^{-10}$ *M* (drug-treated cells) or in the absence of the drugs (control cells)[12].

The contents of free cholesterol, free fatty acids and phospholipids in lymphoid cells from three groups of mice (normal mice, mice bearing with ascitic carcinoma at 10 days and animals bearing with solid tumors at 14 days) were presented in Figure 1. As can be seen, the levels of each lipid fraction in lymphoid cells from thymus, spleen