resistant to treatment with trypsin (2 mg/ml, 60 min, 25 °C). Egg mass extract did not lyse freeze-dried *Micrococcus luteus* cells. The absence of lytic properties was also shown by the fact that the addition of antibacterial factors to a bacterial culture did not cause a reduction of turbidity. *Aplysia* antimicrobial factors are quite different from the lysozymes found in many species of marine invertebrates[4,5] in the following characteristics; their heat-lability, high molecular weight, and lack of lytic properties. These properties of the agents are similar to those of the antibacterial factor against Gram-negative bacteria found in the coelomic fluid of the marine annelid *Glycera dibranchiata*[6,7]. The extract of *Aplysia* egg mass also possesses antitumor activity against various murine tumor cell lines both in vivo and in vitro. Details will be presented elsewhere.

Table 2 summarizes the distribution of antibacterial factors against *B. subtilis* and agglutinins in the various tissues of *A. kurodai*. Albumen gland showed potent antimicrobial activity but no hemagglutinating activity. 2 out of 4 mucous gland samples showed weak antibacterial activity, probably due to contamination with albumen gland. Ovo-testis showed high hemagglutinating activity but no antibacterial activity. Hypobranchial gland also gave weak agglutinating activity. On the other hand, hemolymph caused stimulation of bacterial growth compared to the control. Neither antibacterial nor hemagglutinating activity was observed in extracts of gill, mantle, foot, or digestive gland. These data suggest that antibacterial factors are produced in the albumen gland of *A. kurodai* and that each egg is coated with antibacterially active albumen before passing down the oviduct to the gonapore. It is of interest from the comparative physiological point of view that the albumen glands of certain snails contain agglutinins[8] and protease inhibitors[9], whereas that of *Aplysia* does not possess agglutinins but does produce antimicrobial proteins.

Agglutinins in the egg mass are unlikely to play a significant role in the defense of the egg mass after laying, since antibacterial factors freed from agglutinins showed an increased specific activity in comparison with the crude extract. It might be possible, however, that agglutinins participate in the defense system of the egg mass by creating an unfavorable environment for bacteria.

Table 2. Distribution of antibacterial factors and agglutinins in *Aplysia kurodai*

	Antibacterial activity*	Hemagglutinating activity**
Egg mass	++	2^8
Albumen gland	++	–
	++	–
	++	–
	++	–
Mucous gland	–	–
	–	–
	+	–
	+	–
Gill	–	–
Mantle	–	–
Foot	–	–
Ovo-testis	–	2^{11}
Digestive gland	–	–
Hypobranchial gland	–	2^4
Hemolymph	–	2^6

* ++, decrease in optical density > 70%; +, 70–50%; ** Agglutinating titer against rabbit erythrocytes.

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0014-4754/84/090947-03$1.50 + 0.20/0

On the binding of steroid sulfates to albumin[1]

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Summary. ^{3}H-Labeled steroid sulfates, sulfate of estrone (E_1S) or dehydroepiandrosterone (DHAS), were dialyzed against delipidated human serum albumin or human plasma in the presence of increasing amounts of competing non-labeled sulfates (DHAS or E_1S). The apparent equilibrium constants (K) of the tracers did not measurably change at concentrations of the non-radioactive sulfates below 10^{-5} mol/l. At higher concentrations, K decreased gradually. The apparent equilibrium constant of ^{3}H-E_1S was diminished by plasma in a similar fashion. It may be concluded that albumin possesses one strong, non-specific binding site. This site, however, does not seem to be utilized for the binding of E_1S in vivo, because of its preferential occupation by other ligands. This may be true for other steroid sulfates as well, depending on their relative abundance in plasma.
Key words. Serum albumin, human; steroid sulfates; steroid binding site.

Steroid sulfates are present in human blood primarily as complexes with albumin[2]. No straightforward information is available on the avidity of albumin binding in vivo. Equilibrium constants estimated from in vitro experiments vary in dependence on the concentration of the sulfate (Scatchard plots are not linear, but concavely curvilinear[2-4]).

It may be deduced from the structure of albumin[5] that the strength of binding can change gradually in the process of occupation of various binding regions, and that the binding potency may even change because of the alterations of the tertiary albumin structure due to binding[6].

In spite of this flexibility of the albumin molecule it may be speculated that – in analogy with other compounds (thyroxin[7], coumarin[8], palmitic acid[9]) – there exists a single strong binding site for steroid sulfates. The aim of the present study was to elucidate whether or not this is the case and if so, whether or not this strong binding site is employed for the binding of steroid sulfates in vivo.

Materials and methods. [6,7-^{3}H]Estrone sulfate (^{3}H-E_1S; 43.0 Ci/mmol) and [7-^{3}H]dehydroepiandrosterone sulfate ([7-^{3}H]3β-hydroxy-5-androstene-17-one 3-sulfate; ^{3}H-DHAS; 24.0 Ci/mmol) were purchased from New England Nuclear, Boston, MA, USA. and purified[10,11] before use. Non-radioactive E_1S[12] was kindly donated by LEO AB, Helsingborg, Sweden, and DHAS was purchased from Makor Chemicals Ltd., Jerusalem, Israel. Their purity was checked by thin-layer chromatography. Human serum albumin (HSA) was 'essentially fatty acid free' (Sigma Co., St. Louis, MO, USA). A 4% solution of HSA (5.8×10^{-4} mol/l) in phosphate buffer (pH 7.4, 0.05 mol/l) was used. A heparinized blood plasma pool was obtained from 19 volunteers during the follicular phase of their menstrual cycle and freeze-dried in 1-ml aliquots. As established by a radioimmunoassay described earlier[10,11], it contained 1.74 nmol/l E_1S and 4.29 µmol/l DHAS. The plasma was reconstituted with water before use. Dialysis bags were prepared from the tubing 18 FO (Union Carbide Corp., Chicago, Ill., USA).

^{3}H-E_1S was dissolved in phosphate buffer (10 ml) together with various amounts of non-radioactive DHAS and subjected to equilibrium dialysis (37 °C, 16 h) against the solution of HSA (1 ml) placed in dialysis bags. Alternatively, ^{3}H-DHAS was mixed with E_1S and dialyzed agains HSA. In another experiment, ^{3}H-E_1S was dialyzed against human plasma mixed with the HSA solution in various proportions. The dialysis procedure was performed according to Westphal[13] with minor modifications described elsewhere[14].

Equilibrium constants (K) were computed on the basis of the following equation:

$$nK \approx \frac{B}{F} \cdot \frac{1}{[P_t]}$$

where n = number of binding sites per protein molecule, B and F are the bound and free fractions of the tracer, and $[P_t]$ = total concentration of protein in mol/l. This approximation is valid for the conditions of the present experiment, i.e., for a large excess of protein[2]. The above equation was further simplified by assuming that the number of used binding sites had the lowest possible value, i.e. n = 1, because of low tracer concentrations (see text to figures).

Results and discussion. In figures 1 and 2 the results of 2 experiments are demonstrated in which equlibrium constants of ^{3}H-E_1S and ^{3}H-DHAS were studied in the presence of increasing amounts of competing non-radioactive sulfates, i.e., DHAS and E_1S, respectively. It follows from these experiments that the equilibrium constants did not change until both non-radioactive sulfates reached a molar concentration of approximately 10^{-5}. After a further increase of the ligand concentrations, the apparent equilibrium constants began to decrease rapidly.

This phenomenon may be accounted for by the presence of one strong binding site in the albumin molecule. After this site has been filled up, other sites of lower avidity are engaged in the binding.

In the experiments demonstrated in figure 1 and 2, it was not possible to distinguish any difference between the behavior of E_1S and DHAS, in spite of the fact that the apparent equilibrium constant of the former compound is twice as high as that of the latter. These facts may be explained by the non-specific character of the strong binding site. This inference is further strengthened by the reasoning that the K-values would

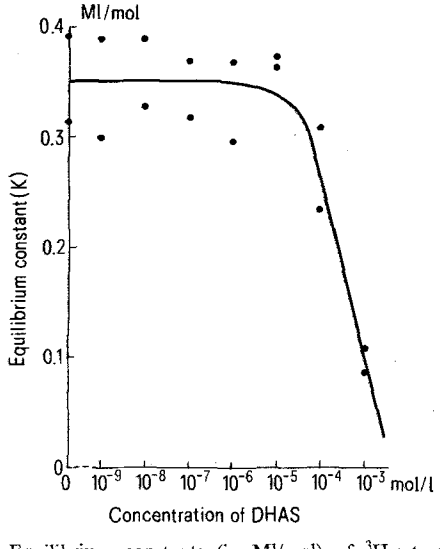

Figure 1. Equilibrium constants (in Ml/mol) of ^{3}H-estrone sulfate (461,200 dpm corresponding to 4.8×10^{-10} mol/l) measured by equilibrium dialysis in duplicate against human serum albumin in the presence of various concentrations of non-radioactive dehydroepiandrosterone sulfate (DHAS).

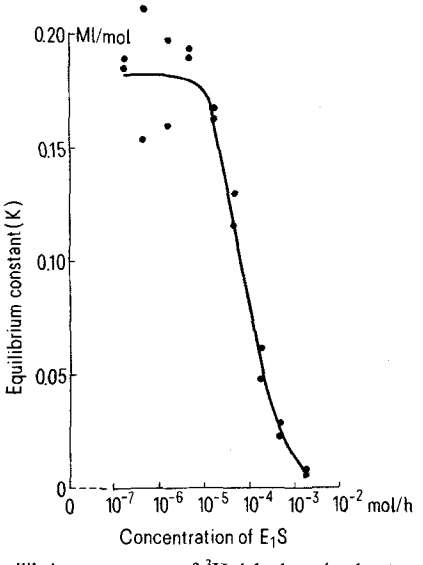

Figure 2. Equilibrium constants of ^{3}H-dehydroepiandrosterone sulfate (435,400 dpm corresponding to 8.2×10^{-10} mol/l) measured in the presence of non-radioactive estrone sulfate (E_1S). See fig. 1 for further details.

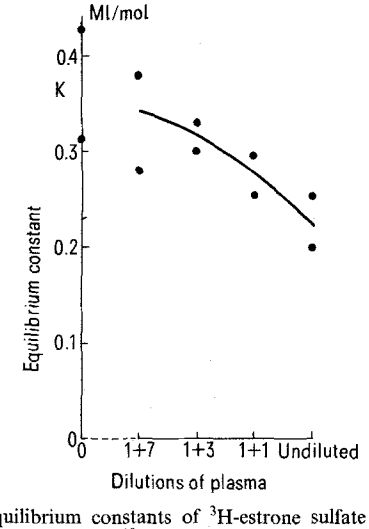

Figure 3. Equilibrium constants of ^{3}H-estrone sulfate (131,400 dpm corresponding to 1.4×10^{-10} mol/l) measured against a human female plasma pool diluted in various proportions with a 4% solution of human serum albumin. See fig. 1 for further details.

remain constant in the present experiments, if the binding site were specific.

It is to be realized that – on the assumption of non-specificity – the above formula for the approximate calculation of K may loose its full validity when the concentration of the cross-reacting steroids approaches or exceeds the concentration of albumin. Even then, however, the conclusion regarding the presence of a single, strong and non-specific binding site can be fully maintained; this conclusion may be reached even if only the behavior of B/F ratios is considered.

The 3rd experiment (fig. 3) showed that undiluted plasma had a similar effect on the binding of ^{3}H-E_1S to albumin as a high concentration (approximately 10^{-4} mol/l) of DHAS (fig. 1). Since the content of endogenous DHAS in the plasma pool under investigation did not reach this level (being 4×10^{-6} mol/l), other ligands must also have participated in filling up the strong binding site.

On the basis of the above experiments it may be concluded that the binding of E_1S (and very probably of other steroid sulfates) is less strong in vivo (having a lower K) than could be inferred from earlier binding studies in the absence of other plasma constituents[2-4]. This seems to be due to the preferential occupation of the strong binding site by other ligands occurring in blood at high concentrations and with high affinities to albumin (fatty acids and other ligands[5]). Consequently it may be expected that the K value in an individual subject and at a given moment is dependent on the concentration of other ligands existing at that time.

1 Acknowledgment. This investigation received financial support from the Special Programme of Research, Development and Research Training in Human Reproduction, World Health Organisation, and from the Ford Foundation.

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0014-4754/84/090949-03$1.50 + 0.20/0

Alanine and aspartate aminotransferases in normal and denervated skeletal muscle

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Summary. Activities of alanine and aspartate aminotransferases are maintained during the first 5 weeks of growth, but decrease subsequently in normal chick gastrocnemii. In sciatectomized muscles, a 5-fold elevation in these enzymes reveals increased utilization of amino acids as a compensatory metabolic support during denervation atrophy.

Key words. Chicken; skeletal muscle; denervated muscle; alanine aminotransferase; aspartate aminotransferase; denervation atrophy; amino acid utilization.

The significance of motor innervation in skeletal muscle growth and metabolism has been emphasized by many workers[2-4]. During postembryonic growth a switchover occurs from a predominantly glycolytic to an oxidative mode of energy generation in normal muscle[5-7], but the denervated muscle registers a loss of glycolytic and oxidative enzymes[8-11], largely due to poorer availability of glycogen. Asotra[10,11] has recently reported that such a metabolic transformation can be regulated by muscle glucose 6-phosphatase. The loss of intrafibrillar lipids by extrusion rather than intense lipase activity in denervated muscle is also well documented[12]. Thus, during growth and especially denervation atrophy, the utilization of substrates other than glycogen and lipids in muscle metabolism is expected to increase. According to Chang and Goldberg[13,14] and Lehninger[15] the normal muscle can utilize the transamination products of amino acids in glycolytic and oxidative metabolism. Whether transamination activities in skeletal muscle are modified upon denervation is not yet known. In this paper we report on the changes in activity levels of alanine and aspartate aminotransferases, which serve as indices of the utilization of the 2 amino acids, in normal and denervated chick gastrocnemii up to 8 weeks of postembryonic growth.

Materials and methods. 1-day-old chicks of *Gallus domesticus* (white leghorn variety) procured from the Government Poultry Farm, Simla were kept on standard chick mash (Hindustan Lever Ltd, Bombay) and water ad libitum under normal laboratory conditions. Chicks were divided into 2 groups; one group served as the normal controls while members of the second were unilaterally denervated on the 5th day of life by excision of a 5 mm piece of sciatic nerve aseptically after s.c. injection of 0.2 ml local anesthetic Xylocaine (5% v/v). Nebasulf powder was sprinkled over the operated area and the wound stitched. Normal and denervated chicks of both sexes were randomly selected and sacrificed by decapitation 1, 2, 3, 4, 5, 6, 7 and 8 weeks postdenervation. At least 18 chicks were used at each stage of investigation and 3 gastrocnemii pars externa, media and interna excised, cleared of fat and connective tissue and weighed. Muscles were homogenized in 0.1 M phosphate buffer, pH 7.4 with 10 strokes using a hand operated glass-and-teflon homogenizer kept on ice. Homogenate was centrifuged at 14,000 × g for 10 min at 4 °C and suitable aliquots of the supernatant used for the bioassay[16] of alanine aminotransferase (EC 2.6.1.2) and aspartate aminotransferase (EC 2.6.1.1) with DL-alanine and L-aspartate as the respective sub-