EFFECT OF CASTRATION AND OF TESTOSTERONE ON ARYLESTERASE ACTIVITY AND PROTEIN CONTENT OF BLOOD PLASMA IN MALE DOGS

By

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ABSTRACT

The arylesterase activity of blood plasma from newborn dogs was about 5 per cent that of normal adult activity values. After birth, the activity increased to reach adult values at the age of about four months. Castration of three mature male dogs resulted in an increase in plasma arylesterase activity. The arylesterase fraction, seen in electropherograms of normal plasma in the pre-albumin region, was absent after castration. On electrophoresis a new protein fraction appeared after castration in the starch gel electropherograms of dog plasma. In addition, a second fraction was faintly visible in normal plasma and increased in amount after castration. Both fractions disappeared when the castrated dogs were treated with testosterone.

Evidence has been presented that the biosynthesis of arylesterase is probably controlled by certain sex hormones (Augustinsson & Olsson 1961 b). Arylesterase activity in pig blood plasma gradually decreased as the boars reached maturity. This decrease in arylesterase activity is due to the effect of male sex hormones on the biosynthesis of active protein and not to a direct inhibitory effect of these hormones on the enzyme. The following observations made on pigs support this view of an enzyme-hormone interaction. After castration of the mature boar, the arylesterase activity increases, reaching after 3 weeks a value close to the maximum activity found briefly before maturity. This activity could again be reduced to the low value when testosterone was administered intramuscularly.

The following report describes similar experiments in dogs. The plasma
esterase pattern of dogs is more complicated than that of pigs and is characterised by at least two arylesterases and two cholinesterases (Augustinsson 1959). In addition to the esterase studies, the change in plasma protein pattern caused by castration was followed by electrophoresis on cellulose columns and starch gel.

**MATERIAL AND METHODS**

**Animals.** The dogs used belonged to a group of Dalmatians which were used in an investigation of hereditary deafness in this breed. Three adult males and four adult females form the basis of this group. Their progeny, six males and eleven females, were investigated for esterase activity several times during the age interval between one and eight or ten months. In addition determinations were made on cord blood from four of these animals. Three mature and two immature males were castrated and treated as indicated below.

**Blood plasma.** Blood samples were collected with heparin without anaesthetia, by puncture of the saphenous vein. The plasma was centrifuged free from all cells including thrombocytes and used for esterase determination. The erythrocytes were washed three times with saline and then haemolysed with distilled water added to blood volume.

**Esterase determination.** The esterase activity was measured by the Warburg technique at 25°C in a bicarbonate-CO₂ buffer containing 33.6 mm NaHCO₃ and 1.0 mm CaCl₂ (for arylesterase) or 1.2 mm MgCl₂ (for cholinesterase), pH 7.4. Phenyl acetate in a final concentration of 10 mM was used as substrate for arylesterase: the same final concentration of butyrylcholine iodide was used for plasma butyrocholinesterase and of acetylcholine iodide for erythrocyte acetylcholinesterase. Corrections were made for spontaneous hydrolysis of substrate and, in certain cases with phenyl acetate as substrate for hydrolysis catalysed by cholinesterase, by carrying out the determination in the presence of prostigmine bromide (final concentration, 0.1 mM).

Esterase activity is expressed in μmoles of substrate hydrolysed per min per ml plasma or erythrocyte haemolysate. In previous reports on these and related enzymes (Augustinsson 1957; Augustinsson & Olsson 1961 a) the esterase activity was expressed in μl CO₂ evolved during 30 min and symbolized by b₃₀; these values can easily be converted to those used in the present report and recommended by the International Union of Biochemistry (1961).

**Electrophoresis.** Electrophoretic separation of plasma proteins was performed on cellulose columns, 1.5 cm × 50 cm, in veronal buffer solution (pH 8.6, I 0.1) at 5–11°C (Augustinsson 1959). For each run, 1.2 ml of plasma was used, the current was 35 mA provided by an applied voltage of 300 V, and the duration of runs 14 to 15 hours. After the completion of electrophoresis, the liquid in the column was displaced at a rate of 10 ml per hour in 1.2 ml fractions. The protein concentration of each fraction was estimated by the modified Folin procedure.

Starch gel electrophoresis was carried out using a generally accepted technique (Bloemendal 1963) with borate-tris buffer, pH 8.6 (0.006 M ± 0.06 M). The voltage applied was 7 V/cm and the duration of runs 3 to 4 hours. The 6 mm thick gel was sliced in three 2 mm thick slices and the middle one was stained for proteins with Amido Black 10B in methanol-water-acetic acid (50:50:10) (Gahne 1961). The bottom slice was stained for esterases by incubation for 30 min at room temperature in a
solution prepared in the following way. Alpha-naphthyl acetate was dissolved in a small volume of acetone and then bicarbonate buffer (for ArE) was added to get a saturated aqueous solution.

Castration and testosterone administration. Male dogs were castrated in the usual manner at the Surgical Clinic of the Veterinary College.

Testosterone propionate (Testodrin, Astra) was injected intramuscularly. The dose was approximately 0.75 mg per kg body weight daily on the days indicated in the figures shown in the next paragraph.

RESULTS

Types of esterases investigated

The main esterases present in dog plasma are arylesterases (ArE) and cholinesterase (ChE). The presence of an esterase, formerly known as «ali-esterase», hydrolysing preferentially aliphatic esters, resistant to physostigmine, and sensitive to organophosphate esters, is still an open question (Augustinsson 1959, 1961). The ArE activity is mainly due to an esterase fraction (ArE I) moving in electrophoresis close to the albumin fraction (cf. Fig. 6). Other fractions able to hydrolyse phenyl acetate have been observed in electrophoresis migrating at a higher rate (a pre-albumin fraction) and at a lower rate (close to the $\alpha_2$-globulins); both these activities are resistant to $iso$-OMPA. The specificity of ArE I of dog plasma is characterised by a high hydrolysis rate of phenyl acetate as compared with that of other phenyl esters.

The ChE present is a butyrylcholinesterase found in electrophoresis between the $\alpha_2$- and $\beta$-globulins (Fig. 6). The esterase fractions in this region are probably not uniform.

Arylesterase activity in dog plasma

The plasma ArE activity of normal dogs, measured with phenyl acetate as substrate, corresponds to about 75 $\mu$moles of substrate hydrolysed per min per ml plasma. Less than one per cent of the activity measured in this way is due to ChE. In newborn dogs, in which ChE is the predominant esterase in the plasma (see next paragraph), the percentage activity due to this enzyme is consequently higher. In these cases the ArE activity was measured in the presence of prostigmine (0.1 mM) in order to inhibit the ChE activity.

All animals used belonged to the same breed and were related to some extent. In only one case was the ArE activity higher than 75. A mature male (dog No. 4), the sire of two dogs studied in detail (Fig. 4), had an ArE activity level of 98. Experimental mating with this male and females with an activity level of 75 did not result in a segregation of different activity phenotypes. From these and other mating experiments we conclude that the ArE activity levels in the mature animals studied were fairly constant and governed by
genetic factors. Further studies on these genetic factors have not been performed in dogs. The genetic control of this enzyme in pigs has been described elsewhere (Augustinsson & Olsson 1961a).

Except for dog No. 4 there were no sex differences in the ArE activity among the group of dogs (nine males and fifteen females) studied in the present investigation. After sexual maturity the activity is fairly constant.

Age variation in arylesterase activity of blood plasma

The ArE activity of the plasma of newborn dogs is very low, less than 5 units. This low activity was observed in blood samples taken from the cord of some newborn animals (not the same animals used in the experiments described below). About 50% of this activity measured against phenyl acetate was due to ChE, evident when the activity was measured in the presence of prostigmine bromide.

After birth the plasma ArE activity increases to reach normal adult values at the age of about 4 months. At this age the dogs have not reached sexual maturity. This starts at about 6 months and is completed at about 8–9 months of age. There seems to be no sex difference in this increase of ArE activity after birth.

**Fig. 1.**

Arylesterase (ArE) and butyrocholinesterase (BuChE) activities of blood plasma in a three-year-old male dog before and after castration, and the effect of testosterone. Esterase activity expressed in μmoles of substrate (phenyl acetate for ArE and butyrylcholine iodide for BuChE) hydrolysed per min per ml plasma. Starch gel electropherograms developed for proteins with amido black; the electropherograms inserted are representative for all samples analysed. Testosterone propionate was administered intramuscularly daily as indicated.
Arylesterase (ArE) activity of blood plasma in two male dogs before and after castration, and the effect of testosterone. Starch gel electropherograms developed for esterase with α-naphthyl acetate. Open circles refer to dog No. 30, filled circles to dog No. 31. See Fig. 1 for further explanations and Fig. 3 for examples of original starch gel electropherograms.

Fig. 2.

Starch gel electropherograms of the plasma of dog No. 30, before (A) and after (B) castration. The samples analysed are the same as those taken at the age of 190 and 201 days (see Fig. 2).

Fig. 3.

Effect of castration on plasma arylesterase activity

Castration of three mature male dogs resulted in an increase in the ArE activity which reached maximum levels in a few days (Figs. 1 and 4). In two not fully matured dogs this effect was not observed (Fig. 2). The abnormally
Arylesterase (ArE) activity of blood plasma in two male dogs before and after castration. Open circles refer to dog No. 23, filled circles to dog No. 27. See Figs. 1 and 2 for further explanations and Fig. 6 for details of electropherograms.

Starch gel electropherograms of the plasma of dog No. 23, before (A) and after castration (B and C), and after testosterone administration (D). The samples analysed are the same as those taken at the age of 248 (A), 253 (B), 269 (C) and 289 (D) days (see Fig. 5).

high level decreased slowly during the following weeks to that before castration. In some cases testosterone propionate seemed to have a lowering effect (Figs. 1 and 2).
Electrophoretic distribution of esterase activity and total protein in blood plasma of an adult male dog (No. 23 of Fig. 4) before (A) and 10 days after castration (B). Column electrophoresis carried out with 1.2 ml of plasma applied on a cellulose column (1.5 cm × 40 cm) barbital buffer, $p_H$ 8.6, $I = 0.1$, 300 V, 35 mA; 14$\frac{1}{4}$ h. Displacement from the column in 1.2 ml fractions. Protein content (dotted line) expressed in relative concentration values (Folin colour). Esterase activity expressed in $b_{30}$ values (Warburg technique) with phenyl acetate and 0.05 ml aliquots from each fraction (except for the pre-albumin fraction, 0.4 ml) for arylesterase (ArE), and with butyrylcholine iodide and 0.4 ml aliquots for butyropholinesterase (BuChE). Starch gel electrophoresis of the fractions indicated, carried out with borate-tris buffer ($p_H$ 8.6; 300 V, 45 mA; 3$\frac{1}{2}$ h). Staining of proteins with amido black.
The esterase patterns obtained by developing starch gel electropherograms of whole plasma with α-naphthyl acetate were the same before and after castration (Fig. 2).

The electropherograms of plasma samples taken before and after castration and obtained by electrophoresis on cellulose columns differed, however, as far as the esterase peak in the pre-albumin region is concerned (Fig. 6). As mentioned above, normal dog plasma contains this ArE fraction. This activity, only a few per cent of the total ArE activity of plasma, was absent after castration. No or only small differences could be observed in plasma ChE activity before and after castration either in the activity level or in the electropherograms.

**Effect of castration on the protein content of plasma**

The protein content of the plasma samples analysed for esterase activity was studied by electrophoresis both on starch gel (Figs. 1–5) and on cellulose columns (Fig. 6). After castration of male dogs, the starch gel electropherograms of the plasma showed one new protein band between the F α_2- and the β-bands (Figs. 2, 3, 5 and 6). This new band appeared about 24 h after castration. In addition, a second band, moving close to the new band and very faintly visible in normal plasma, increased in density after castration. Both these bands disappeared when the castrated dogs were injected daily with testosterone propionate. Such an effect of castration on the protein pattern of blood plasma has not been previously described.

**Discussion**

There are great differences in esterase patterns of blood plasma among mammalian species. These differences make it difficult to apply results obtained with one species to another. The presence of arylesterase in the plasma, however, is a characteristic feature of all mammals studied (Augustinsson 1959). Although these enzymes have certain common properties, they exist in various forms. Two or more active fractions with arylesterase activity were obtained by electrophoresis of human, rabbit, rat and dog plasmas. These forms showed similar substrate specificity or differed only slightly in hydrolysis rates of various aromatic esters. Differences in sensitivity to certain metal cations were more pronounced.

The complexity of dog plasma arylesterase has not been fully investigated but there are at least two entities, both running close to the albumin fraction in electrophoresis. The two active arylesterase fractions may have different physiological functions, the study of which is part of our present investigation on these enzymes.

The plasma of newborn dogs has a very low arylesterase activity which
increases to normal adult values at the age of about four months. Similar observations have recently been made on human subjects (Augustinsson & Brody 1962). As in dogs, human subjects have two or more types of arylesterase in their plasma – ArE I, ArE II and probably an »albumin-esterase«. The increase in activity after birth seems to be mainly due to ArE I, which is the main constituent of this esterase type in human subjects and dogs (Augustinsson & Barr 1963). It is suggested that in dogs too the main arylesterase fraction (ArE I) is responsible for the age variation and that the low activity at birth is due to the other types. ArE II or »albumin-esterase« or both.

In newborn pigs and calves the plasma has no arylesterase activity (Augustinsson & Olsson 1961 a). These species have only one type of plasma arylesterase (ArE I) and this is absent in the plasma at birth. This esterase, therefore, cannot pass from mother to offspring in utero. The low activity of arylesterase in dog plasma at birth can probably be explained in the same way.

As far as the biosynthesis of arylesterase is concerned, recent experiments with pigs have demonstrated that certain male sex hormones inhibit the production in vivo of active arylesterase (Augustinsson & Olsson 1961 b). The results obtained in dogs support this view of enzyme-hormone interaction in the biosynthesis of arylesterases. The effect of castration of male dogs and the subsequent treatment with testosterone, however, were not so conclusive as in the experiments with boars. The complexity of arylesterase constituents in dog plasma compared with the more uniform arylesterase fraction in pig plasma might be an explanation for this difference. Undoubtedly, however, the formation of active protein is influenced by male sex hormones even in dogs. The physiological significance of this interaction in arylesterase biosynthesis is uncertain and is being further investigated in connection with studies on the site of esterase formation in other mammalian species.

The new protein band(s) observed on starch gel electrophoresis of plasma from castrated male dogs is probably related to the transferrins. Whether these fractions are dissociation products of normal globulins or of non-plasmatic origin was not further investigated. This new plasma protein appeared very rapidly after castration. A direct effect of sex hormones on this phenomenon is obvious from the results presented but its physiological significance is doubtful.

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REFERENCES


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