

## Frequency distribution of different types of atrial receptors

Animals	Anaesthesia	No. of animals	No. of atrial receptors		Inter-mediate	A:B ratio
			A	B		
Cats	Nembutal or chloralose	14	35	61	11	1:1.8
Dogs	Nembutal	20	3	47	2	1:16
Monkeys	Nembutal or chloralose	8	0	8	0	0:8
Rabbits	Nembutal or urethane	20	0	0	0	0:0

The possibility of atrial afferent fibres in the laryngeal communicans has to be considered in view of the observations of CASTENFORS, KNUTTSON and SJOSTRAND<sup>10</sup> in rats. Though the laryngeal communicans in the rabbits has not been screened in the present study, ANDREW<sup>11</sup> did not find in it any fibres with a cardiac rhythm. This makes it unlikely that there are atrial afferent fibres coursing via this route.

This study does not rule out the possibility of atrial receptors with non-medullated afferent connections. This is because, in that case, the activity would not have the same relation to the ECG as when the afferent fibres are medullated. The slower conduction velocity of the non-medullated fibres would make the type B activity liable to be mistaken for arterial baroreceptor activity. But AARS<sup>12</sup> found that afferent fibres with a baroreceptor pattern of activity in the depressor nerve of the rabbit had conduction velocities in the medullated fibre range.

CLEMENT, PELLETIER and SHEPHERD<sup>13</sup> showed reduced renal sympathetic activity in response to dextran infusion and increased activity in response to bleeding in rabbits. These responses were abolished by vagotomy or cooling the vagi to 2–5°C. They suggested the possible involvement of receptors in the low pressure system, implying the classical atrial receptors. From our results, it seems more likely that their responses were mediated through some other afferent fibres, e.g. non-medullated vagal fibres from the heart or elsewhere which would also be blocked at a temperature of 2–5°C (PAINTAL<sup>14</sup>).

In conclusion, our results suggest that the type A and type B atrial receptors are different functional categories.

**Summary.** The difference in the frequency distribution of the A and B types of atrial receptors in different laboratory animals suggests that the two types belong to functionally separate categories.

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<sup>10</sup> J. CASTENFORS, E. KNUTTSON and T. SJOSTRAND, *Acta physiol. scand.* **84**, 355 (1972).

<sup>11</sup> B. L. ANDREW, *J. Physiol., Lond.* **125**, 352 (1954).

<sup>12</sup> H. AARS, *Acta physiol. scand.* **83**, 335 (1971).

<sup>13</sup> D. L. CLEMENT, C. I. PELLETIER and J. T. SHEPHERD, *Circulation Res.* **31**, 824 (1972).

<sup>14</sup> A. S. PAINTAL, *J. Physiol., Lond.* **193**, 523 (1967).

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### Study on Sex-Specific Transferrin Polymorphism and on the Identification of Transferrins by Radioactive Labelling

The electrophoretic separation of mouse serum has shown the genetic polymorphism of serum- $\beta$ -globulin<sup>1,2</sup>. By labelling the proteins with <sup>59</sup>Fe, the  $\beta$ -globulin fractions were autoradiographically identified as transferrin bands<sup>3</sup>. The transferrin locus (Trf), which shows the genetic polymorphism, consists of 2 alleles (Trf<sup>a</sup> and Trf<sup>b</sup>). They can be distinguished by the anionic electrophoretic migration of the  $\beta$ -globulins. Trf<sup>a</sup> is represented by the 3 faster moving bands, Trf<sup>b</sup> by the 3 fractions moving more slowly.

In order to investigate the transferrin polymorphism, we separated the serum proteins by means of polyacrylamide gel electrophoresis, similar to the method of ABRAHAM et al.<sup>4</sup> (Modifications: Length of the gel 7.5 cm; total monomer concentration 6.08/100 ml; grade of polymerization 0.86%).

For this study a non-inbred mouse population was used<sup>5</sup>. The population was completely homozygote with respect to the transferrin locus (Trf<sup>b</sup>/Trf<sup>b</sup>). However, in the  $\beta$ -globulin fractions some sex-specific differences could be observed.

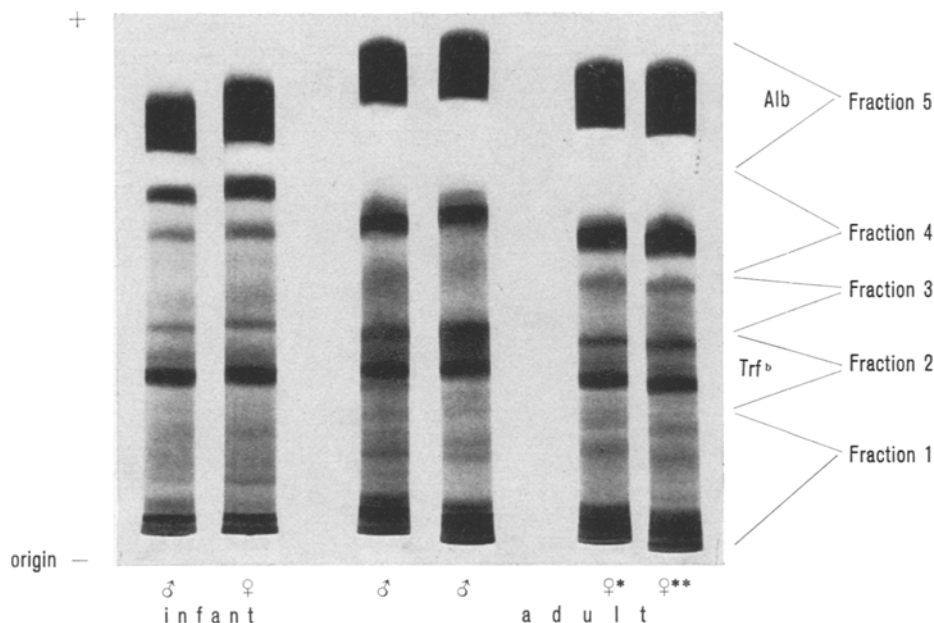
<sup>1</sup> B. L. COHEN, *Genet. Res. Cambridge* **1**, 431 (1960).

<sup>2</sup> G. C. ASHTON and A. W. H. BRADEN, *Austr. J. biol. Sci.* **14**, 248 (1961).

<sup>3</sup> E. R. GIBLETT, C. G. HICKMAN and O. SMITHIES, *Nature, Lond.* **183**, 1589 (1959).

<sup>4</sup> K. ABRAHAM, K. SCHÜTT, I. MÜLLER and H. HOFFMEISTER, *Z. klin. Chem. klin. Biochem.* **8**, 92 (1970).

<sup>5</sup> J. H. WENIGER, P. HORST, D. STEINHAUF, F. MAJOR, M. WOLF and E. S. TAWFIK, *Z. Tierzücht. Zücht Biol.* **91**, 265 (1974).



Electrophoretic patterns of mouse serum proteins. \*: gravid; \*\*: non-gravid.

The electrophoretic separation of the serum proteins of infant and adult mice showed that the transferrin bands could not be seen in 3-day-old animals. The bands did not appear until the mice were 10–12 days old. At the age of about 30 days, when the animals were already sexually mature, a difference with regard to the transferrin fractions between male and female animals was found (Figure).

In this dimorphism the 1st and 3rd transferrin bands became very marked in the male serum, while in the female serum samples the first band was considerably lighter. The densitometric measurement of the separated protein fractions of 115 male and 115 female mice revealed a significant difference ( $p < 0.001$ ) between the protein content of the 3 Trf-bands in the male (18.38% of the total protein) and in the female animals (17.35%).

Moreover, an additional fraction above the transferrin bands was observed amongst the females only. The protein pattern was the same in gravid and non-gravid animals. In the inbred mice populations BALB/C/A/BOM, CBA/J, DE/J, C<sub>57</sub>BL/6J and AU/SsJ this fraction could not be found.

Test for the differences in the average counting rate in 5 fractions

	Fractions				
	1	2	3	4	5
Average counting rate per 1% protein	5.96	7.11	4.74	4.80	4.75
Differences between the fractions					
2	+				
3	+	+			
4	+	+	—		
5	+	+	—	—	

+ Significant at  $p < 0.001$ ; —, not significant.

The question whether the additional band is part of the transferrin or a completely different component was investigated by labelling the proteins with  $^{59}\text{Fe}$ . Here 32 randomly selected 60-day-old non-gravid females from the same non-inbred population were used. In order to label the serum with  $^{59}\text{Fe}$ , 15  $\mu\text{l}$  of serum from each sample were mixed with 5  $\mu\text{l}$  of  $^{59}\text{Fe}$  (III) citrate solution. The mixture was then incubated at 4°C for 12 h. From each of the labelled serum samples, 5  $\mu\text{l}$  containing 0.02  $\mu\text{Ci}$   $^{59}\text{Fe}$  were taken for the electrophoretic separation.

After the separation, the protein content in each fraction, expressed as the percentage of the total protein content, was determined in a densitometer. Then the gel column was cut into 5 parts (Figure), and the counting rate of the  $^{59}\text{Fe}$ -activity in each protein fraction was measured by means of a  $2 \times 2$  in. NaJ(Tl) well-type crystal connected to a single-channel analyzer. The result was expressed as counting rate per percentage of the protein content (Table).

The highest counting rate per 1% protein was found in fraction 2, which contained the transferrins. It is interesting to note that in all the other protein fractions too relatively high counting rates were obtained. This indicates that in addition to the transferrins, other proteins are also able to bind iron ions. As slight differences in the distribution of radioactivity are very difficult to determine in quantity by autoradiography, the detection of radioactivity by means of a scintillation crystal proved to be the more favourable method.

In order to find out how each fraction differed from the others regarding its  $^{59}\text{Fe}$ -activity, the range-test<sup>6</sup> in combination with least squares analysis of variance<sup>7</sup> was carried out. The results are shown in the Table.

<sup>6</sup> A. MUDRA, *Statistische Methode für Landwirtschaftliche Versuche* (Paul Parey, Berlin and Hamburg 1958).

<sup>7</sup> W. R. HARVEY, A. R. S. 20–8, U. S. Department of Agriculture (USDA) reprint, July 1968, p. 157.

In fraction 1 the electrophoretic starting point of the labelled serum was included. This may account for the fact that here the  $^{59}\text{Fe}$ -activity was significantly higher than in the fractions 3, 4 and 5. Fraction 2, which contained the transferrins, had the highest counting rate per 1% protein and was significantly different from all the other fractions. Fraction 3, the object of our investigation, had the same activity per protein content as fraction 4 and 5.

From these results it could be concluded that the sex-specific fraction 3 in the female animals was not a part of the transferrin bands.

**Summary.** Sex-specific differences with regard to the intensity of transferrin bands were observed in a non-inbred adult mouse population after separation of the serum proteins by polyacrylamide gel electrophoresis. Amongst the female animals, an additional protein fraction was found just above the position of the transferrin

bands. By means of a tracer method, using  $^{59}\text{Fe}$ -labelling, it could be shown that the additional fraction is not a part of the transferrin bands.

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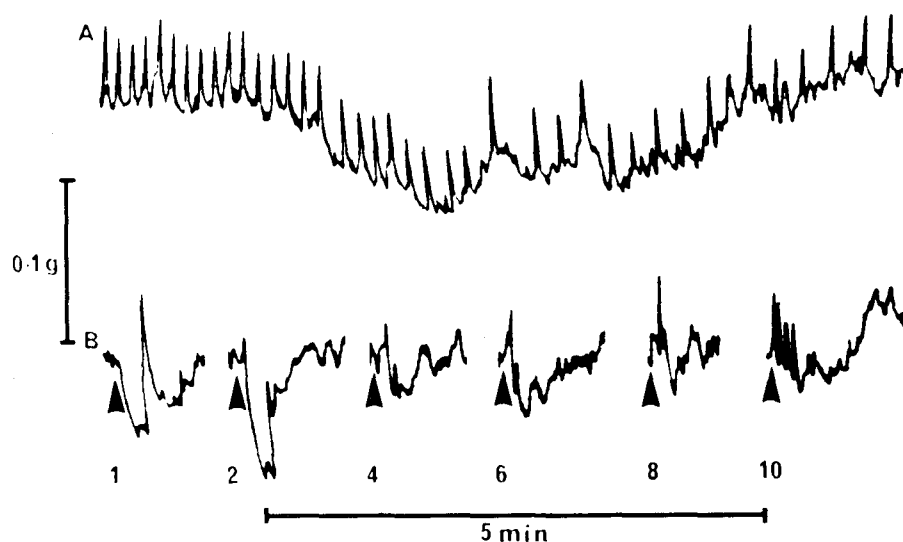
## Autonomic Control of Renal Portal Blood Flow in the Domestic Fowl

SPERBER<sup>1</sup> was the first to demonstrate a functional renal portal system in the domestic fowl; his observations have latterly been extended<sup>2</sup>. The integrity of this system depends on the activity of the renal portal valve which lies at the junction of the renal vein, external iliac vein and inferior vena cava<sup>2</sup>. This valve is composed largely of circularly arranged smooth muscle cells that have been shown, by histochemical techniques, to receive a dense noradrenergic and cholinergic innervation<sup>3-5</sup>. Pharmacological observations on the renal portal valve from the turkey indicate that acetylcholine has excitatory and adrenaline has inhibitory effects<sup>6</sup>. However, there have been no physiological studies on the nervous control of the smooth muscle of the renal portal valve; in the present account, such a study is reported.

**Materials and methods.** 12-week-old White Leghorn chicks were decapitated under light ether anaesthesia and the renal portal valve from the left side was removed intact. The valve was then slit open and mounted in an organ bath in such a way that contraction of the smooth muscle cells was recorded as a change in length. The tissue was bathed in a physiological saline solution at 37 °C

gassed with 95% O<sub>2</sub>, 5% CO<sub>2</sub>. The nerve fibres within the renal portal valve were stimulated through a pair of platinum wire electrodes arranged either side of the tissue.

**Results.** The majority of preparations exhibited rhythmic spontaneous activity, superimposed on slow changes in tone (Figure A). This activity appeared to be myogenic since it was unaffected by autonomic drugs. The response of the renal portal valve to nerve stimulation depended on stimulus frequency. At low frequencies (1–2 Hz) there was a marked relaxation of the tissue with little excitatory effect (Figure B). However, with higher stimulus frequencies (4–10 Hz) there was an initial excitatory response followed by a prolonged relaxation. The excitatory re-



A) Spontaneous activity in the renal portal valve. Note the frequent spontaneous contractions superimposed on the slow changes in tone. B) Responses of the renal portal valve to nerve stimulation (60 V strength, 0.2 msec duration for 10 sec periods) at frequencies of 1 to 10 Hz. Note the predominant relaxatory response at low frequencies and the initial contraction seen with higher stimulus frequencies.

<sup>1</sup> I. SPERBER, *Zool. Bidr. Upps.* 27, 429 (1948).

<sup>2</sup> A. R. AKESTER, *J. Anat.* 101, 569 (1967).

<sup>3</sup> A. R. AKESTER and S. P. MANN, *J. Anat.* 104, 241 (1969).

<sup>4</sup> S. DOLEZEL and K. ZLÁBEK, *Z. Zellforsch.* 100, 527 (1969).

<sup>5</sup> T. BENNETT and T. MALMFORS, *Z. Zellforsch.* 106, 22 (1970).

<sup>6</sup> B. R. RENNICK and H. GANDIA, *Proc. Soc. exp. Biol. Med.* 85, 234 (1954).