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SERUM PROTEINS IN MINK WITH ENDOTOXIN-INDUCED AMYLOIDOSIS AND INFECTIOUS PLASMACYTOSIS

By

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MOHN, S. F. and K. NORDSTOGA: *Serum proteins in mink with endotoxin-induced amyloidosis and infectious plasmacytosis.* Acta vet. scand. 1975, *16*, 288—296. — The serum proteins in Sapphire mink from the experimental and control groups in 2 endotoxin experiments and in a group of normal mink of the Standard type, were separated electrophoretically on cellulose acetate membranes.

In experiment No. 1, in which the experimental mink were given repeated injections of endotoxin, and the controls were untreated, significantly increased total protein and significantly decreased albumin concentrations in the experimental group compared to the normal group were demonstrated. The concentration of the alpha$_1$-globulin was significantly elevated and the alpha$_2$-globulin significantly reduced in the experimental and the control groups compared to the normal group. Significant differences between the gamma-globulin concentrations in the various groups were not found.

In experiment No. 2, all the animals were inoculated intraperitoneally with a crude tissue suspension containing the plasmacytosis agent 10 days before the experimental animals received the first of a series of injections with endotoxin. Significantly increased concentrations of total protein and gamma-globulin and significantly decreased albumin concentrations were, compared with the normal group, demonstrated in sera collected from the experimental group on the 94th, 201st and 254th day after the first injection of endotoxin. In the control group, compared with the normal group, significantly elevated concentrations of total protein, alpha$_2$-, beta- and gamma-globulins and significantly reduced albumin- and alpha$_1$-globulin were found but only in the second set of samples, while significantly decreased albumin- and significantly increased gamma-globulin concentrations were found in the third set.

The results showed no significant differences between the gamma-globulin concentrations or between the other serum fractions in the experimental and the control groups in the 2 experiments. A possible explanation may be that there is no direct interrelationship between hypergammaglobulinaemia and amyloidosis, and that a common basic mechanism may stimulate related stem cells which thereafter differentiate in different ways.

mink; serum proteins; endotoxin; amyloidosis; plasmacytosis.

Prolonged endotoxin treatment in mice (*Barth et al.* 1969) and mink (*Nordstoga* 1972) is followed by amyloidosis, the response being influenced by strain differences in the mice. Infectious plasmacytosis in mink is a slowly progressive disease in which auto-immune mechanisms are considered to play an important role, and is invariably accompanied by hypergammaglobulinaemia and, relatively often, also by amyloidosis. Mink which are homozygous for the aleutian gene are predisposed for the infection (*Obel* 1959, *Trautwein* 1964, *Saison et al.* 1966, *Karstad* 1967).

Plasma cell neoplasms are, in other species, sometimes accompanied by amyloidosis (*Ebbesen & Rask-Nielsen* 1967, *Lehner & Rosenoer* 1968, *Shepard et al.* 1972), while Sapphire mink or plasmacytotic animals do not seem to develop amyloidosis more easily than other mink after endotoxin treatment (*Nordstoga* 1972).

The present report constitutes part of an endotoxin study, in which the aim was to investigate whether prolonged endotoxin treatment, in addition to development of amyloidosis, also caused changes in the composition of serum proteins in otherwise healthy, and in plasmacytotic mink.

MATERIAL AND METHODS

Normal group

Sera from 34 healthy, adult mink of the Standard type representing both sexes were examined.

Experimental groups

Endotoxin. Escherichia coli 026:B 6 endotoxin (Difco Laboratories, Detroit, Mich., USA) was dissolved in saline and injected subcutaneously.

Experimental animals. Female young Sapphire mink were used. Experiment 1 comprised 50 kits which were equally divided into an experimental group, which received multiple injections of endotoxin, and an untreated control group (*Nordstoga & Næss* 1973). Blood samples were collected at the killing, 126 days after the first endotoxin injection.

In experiment 2 all animals were injected with a suspension of mink organs containing the agent of plasmacytosis; the animals were allocated to 2 equal groups; the experimental groups were given multiple injections of endotoxin and blood samples collected on the 94th, 201st and 254th day after the beginning of the experiment. The experimental procedures have been given in greater detail in previous reports (*Nordstoga & Næss, Nordstoga* 1973).

Total protein concentrations

Standard refractometric procedures were followed (Refractometer, Cat. No. 33.45.87, Baush & Lomb).

Electrophoresis

Separation of the serum proteins was carried out on cellulose acetate membranes in a Microzone Electrophoresis Cell (Model R-101, Beckman). The runs were performed in barbital buffer, pH 8.6, ionic strength 0.075 (Beckman B-2 Buffer) at 250 v for 20 min. (*Levine* 1965).

Scanning

The membranes were scanned in a densitometer (Model 52-C, Photovolt) and the relative concentrations of each serum fraction were measured electronically in an integrator (Model 49-A, Photovolt).

Calculations

The total protein and serum fraction concentrations in each group are expressed as $\bar{x} \pm s$ where x represents the measured or calculated amount of protein in each sample and s the standard deviation. The data were treated statistically at the confidence level of 99 % ($P = 0.01$).

In order to test the reproducibility of the separation method, 24 electrophoretic runs were made using 1 serum sample from a healthy adult mink. The following mean relative concentrations (%) were found: Albumin 48 ± 4, alpha$_1$-globulin 5 ± 1, alpha$_2$-globulin 10 ± 2, beta-globulin 21 ± 1 and gamma-globulin 17 ± 3.

RESULTS

Four experimental animals and 2 controls in experiment 1 died during the experimental period, whereas the deaths in experiment 2 were 22 and 15 animals, respectively. Fifteen experimental animals in experiment 1, and 24 experimental mink and 3 controls in experiment 2 developed amyloidosis (*Nordstoga & Næss* 1973, *Nordstoga* 1973). Estimations of the mean concentrations of total protein and serum fractions are given in Fig. 1, and the electrophoretic patterns of 1 experimental mink (No. 26 of experiment 2, total protein 9.9 g/100 ml) and of 1 healthy mink (No. 900 T, total protein 7.1 g/100 ml) are shown in Fig. 2; the sera being separated in 1 albumin- and 4 globulin fractions which were designated alpha$_1$, alpha$_2$, beta and gamma.

The mean absolute concentration values in normal and experimental groups are compared and the significances of the differences listed in Table 1.

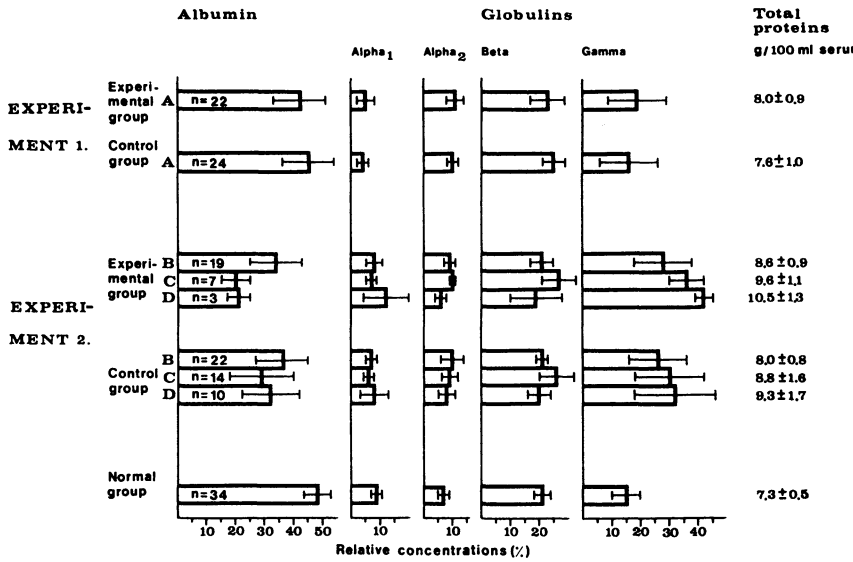

Figure 1. Mean concentration values of serum fractions and total proteins in the experimental and control groups of experiment 1 and experiment 2 and in the normal group. The experimental animals in both experiments received repeated injections of endotoxin. All animals in experiment 2 were inoculated intraperitoneally with a crude tissue suspension containing the agent of plasmacytosis 10 days before the experimental animals received the first injection of endotoxin.

A = sera collected on the 126th day after the first injection of endotoxin.
B = „ „ „ „ 94th „ „ „ „ „ „ „
C = „ „ „ „ 201st „ „ „ „ „ „ „
D = „ „ „ „ 254th „ „ „ „ „ „ „

DISCUSSION

Changes in the serum proteins, including hypergammaglobulinaemia, are, at times, present in amyloidotic animals, also in cases unrelated to plasma cell tumours, although hypergammaglobulinaemia is not a prerequisite for amyloidosis (*Calkins* 1968, *Muckle* 1968, *Willerson et al.* 1969, *Koenig & Rigdon* 1970).

In experiment 1 in this investigation the total protein was increased and the albumin fraction decreased when compared with the normal group, whereas there was no difference between the experimental animals and the controls. This feature is characteristic for the changes in the serum proteins associated with infectious plasmacytosis, and may possibly be explained by the circumstance that all the animals were of the Sapphire type and

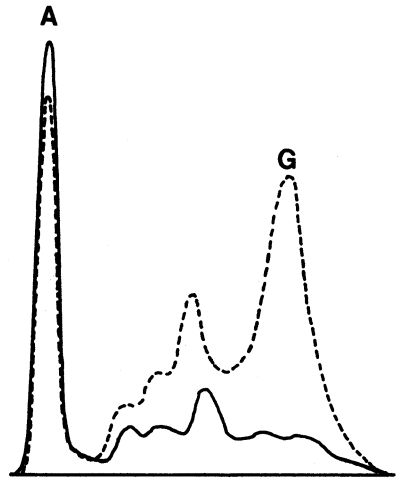

Figure 2. Electrophoretic patterns of sera from the healthy mink No. 900 T (drawn line) and from mink No. 26 in the experimental group of experiment 2 (broken line).
A = albumin fraction. G = gamma-globulin fraction. The globulin fractions between A and G are alpha$_1$, alpha$_2$ and beta.

had a high incidence of plasmacytosis. There were, however, no significant differences in the gamma-globulins between these groups; the alpha$_1$ fraction was significantly decreased in the experimental and control groups, if compared with the normal group, whereas the reverse situation was observed for the alpha$_2$ fraction.

The most prominent feature in the serum protein pattern in experiment 2 is the evident elevation in the total protein and the highly elevated gamma-globulins. These phenomena, which are statistically significant when the experimental animals are compared with the normal groups, included all 3 samplings. Concurrently, there is an obvious drop in the albumin fraction. These alterations are easily explicable as a result of plasmacytosis, as such changes are almost invariably present in plasmacytotic mink.

Prolonged endotoxin treatment accelerated the progress of plasmacytosis and there also seemed to be a slight increase in the gamma-globulins of the experimental group compared with the controls. This increase was, however, not significant. Neither was there significant difference between the control and the nor-

Table 1. Differences between absolute concentrations of total proteins and serum fractions in experimental and control groups in experiment 1 and experiment 2 and the normal mink group.

Experiment no.	Samples collected	Groups compared	Differences between mean absolute concentration values, g/100 ml serum					
			total protein	albumin	alpha$_1$-globulin	alpha$_2$-globulin	beta-globulin	gamma-globulin
1	A[1])	Experimental — Normal	0.7 **	— 0.1 **	— 0.2 **	0.3 **	0.3 n.s.	0.4 n.s.
	A	Control — Normal	0.3 n.s.	— 0.3 n.s.	— 0.3 **	0.3 **	0.5 **	0.2 n.s.
	A	Experimental — Control	0.4 n.s.	0.2 n.s.	0.1 n.s.	0.0 n.s.	— 0.2 n.s.	0.2 n.s.
2	B[2])	Experimental — Normal	1.3 **	— 0.8 **	0.1 n.s.	0.3 **	0.3 n.s.	1.4 **
	C[3])	„	2.3 **	— 1.7 **	0.1 n.s.	0.4 **	1.1 **	2.4 **
	D[4])	„	3.2 **	— 1.6 **	1.0 n.s.	0.0 n.s.	0.6 n.s.	3.1 **
	B	Control — Normal	0.7 n.s.	— 0.6 n.s.	— 0.1 n.s.	0.3 n.s.	0.2 n.s.	1.0 n.s.
	C	„	1.0 **	— 1.0 **	— 0.1 **	0.2 **	0.5 **	1.4 **
	D	„	2.0 n.s.	— 0.6 **	0.1 n.s.	0.3 n.s.	0.4 n.s.	1.9 **
	B	Experimental — Control	0.6 n.s.	— 0.2 n.s.	0.2 n.s.	0.0 n.s.	0.1 n.s.	0.4 n.s.
	C	„	1.3 n.s.	— 0.7 n.s.	0.2 n.s.	0.2 n.s.	0.6 n.s.	1.0 n.s.
	D	„	1.2 n.s.	— 1.0 n.s.	0.9 n.s.	— 0.3 n.s.	0.2 n.s.	1.2 n.s.

**: Significant difference ($P \leqq 0.01$)
n.s.: No significant difference ($P > 0.01$)
[1]) A: 126 days after the first injection of endotoxin.
[2]) B: 94 days after the first injection of endotoxin.
[3]) C: 201 days after the first injection of endotoxin.
[4]) D: 254 days after the first injection of endotoxin.

mal group as for the total protein, except at the second sampling. This last mentioned finding may possibly be interpreted as an indication of an endotoxin-induced elevation of the total protein. However, no corresponding elevation could be observed in the experimental group when compared with the controls.

Barth et al. (1969) reported that casein used in their experiments exhibited endotoxin activity, and suggested that the ability of casein to induce amyloidosis in mice may depend on endotoxin contamination; they recognized also, however, strain differences in the immunoglobulin response to prolonged treatment with endotoxin and concluded that the immunoglobulins did not play a direct role in the amyloidogenesis in their experiments. Our findings seem to parallel their conclusion in this respect. It is a common experience that the levels of gamma-globulins continue to increase throughout the development of infectious plasmacytosis, and that amyloidosis occurs in longstanding cases of plasmacytosis, with advanced tissue involvement. It was, therefore, somewhat surprising that no significant differences in the gamma-globulins could be established between the experimental and the control groups in experiment 2, as the plasmacytotic lesions were considerably less in the latter group, in which 3 out of 10 surviving animals were judged as negative on histo-pathological examination (*Nordstoga* 1973). It is, however, also obvious that only a minority of animals affected by infectious plasmacytosis develops amyloidosis. A possible explanation may be that, although hypergammaglobulinaemia and amyloidosis sometimes occur concurrently, there is no direct interrelationship between these phenomena, and that a common basic mechanism may stimulate related stem cells which thereafter differentiate in different ways (*Willerson et al.*).

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SAMMENDRAG

Serumproteiner hos mink med endotoksin-indusert amyloidose og plasmacytose.

Serumproteinene i safirmink fra forsøks- og kontrolgruppene i to forskjellige endotoksinforsøk samt i en gruppe normale standardmink er separert elektroforetisk på celluloseacetatmembraner. Proteinene kunne skilles i en albuminfraksjon og 4 globulinfraksjoner som er betegnet alfa$_1$, alfa$_2$, beta og gamma. Differansene mellom de absolutte

gjennomsnittskonsentrasjoner av totalprotein og de enkelte serumfraksjoner i forsøks- og kontrollgruppene og i normalgruppen er beregnet og sammenlignet statistisk (Tabel 1).

I forsøk 1, hvor forsøksdyrene fikk gjentatte injeksjoner med endotoksin og kontrolldyrene forble ubehandlet, ble det påvist signifikant forøket konsentrasjon av totalprotein og nedsatt albuminkonsentrasjon i forhold til normalgruppen. Konsentrasjonene av alfa$_1$-globulin var signifikant høyere og av alfa$_2$-globulin signifikant lavere både i forsøks- og kontrollgruppen sammenlignet med normalgruppen, mens signifikante forskjeller i gamma-globulinkonsentrasjonene mellom de forskjellige grupper ikke kunne påvises.

I forsøk 2, hvor alle dyrene ble podet med en vevssuspensjon inneholdende plasmacytoseagens 10 dager før første endotoksininjeksjon av forsøksdyrene, var signifikant forøkete totalprotein- og gamma-globulinkonsentrasjoner og signifikant nedsatt albuminkonsentrasjon i sera fra forsøksgruppen uttatt 94, 201 og 254 dager etter første endotoksininjeksjon sammenlignet med normalgruppen, de mest fremtredende forandringer. I kontrollgruppen var det ingen signifikant forskjell i noen av fraksjonene i forhold til normalgruppen ved første prøvetagning, mens det ved andre prøvetagning ble funnet signifikant forøket totalprotein-, alfa$_2$-, beta- og gamma-globulinkonsentrasjoner og signifikant nedsatte albumin- og alfa$_1$-konsentrasjoner. Ved tredje prøvetagning var det signifikant mindre albumin og signifikant høyere gamma-globulin i kontrollgruppen sammenlignet med normalgruppen. Det kunne ikke påvises signifikante forskjeller ved noen av de tre prøvetagningene mellom fraksjonene i forsøks- og kontrollgruppene.

De foreliggende resultater viser at det ikke er påvist signifikante forskjeller verken mellom konsentrasjonene av gamma-globulin eller mellom de andre serumproteinfraksjonene i forsøks- og kontroll-gruppene i de to forsøkene. En mulig forklaring på dette kan være at det ikke er noen direkte forbindelse mellom hypergammaglobulinemi og amyloidose, men at felles mekanismer kan stimulere beslektede stamceller som deretter differensieres på ulike måter.

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