Effect of Prolonged Glucocorticoid Administration on Lipemia, Coagulation and Thrombosis in Rat* (32935)

S. RENAUD[1] AND J. G. LATOUR[2]

Montreal Heart Institute and Department of Pathology, University of Montreal, Montreal, Canada

Recently (1) we reported that the administration of glucocorticoids for a 3- to 7-day period to hyperlipemic rats, resulted in a marked protection against the phlebothrombosis initiated in these animals by intravenous injection of a gram-negative bacteria endotoxin. This preventive effect could be related to a marked prolongation of the plasma clotting time and to a considerable increase in the precentage of α lipoproteins, but not to a stimulation of the fibrinolytic activity. It was later shown (2) that the effect of hydrocortisone administration on the lipoproteins, clotting time, and thrombosis was probably due, at least in part, to an increase in the serum level of albumin. In the studies mentioned above, the glucocorticoids were administered at pharmacologic doses and for short periods of time. Before glucocorticoids could be considered for pactical applications in thrombosis, it is at maintenance dosage and after prolonged administration that the beneficial effects should be observed experimentally. The aim of the present experiments was to determine whether under such conditions, a protective effect on thrombosis could still be demonstrated. To this end, triamcinolone, a potent glucocorticoid, was included in the hyperlipemic diet of rats, at low dosage, for 4 weeks, various blood parameters being determined, and large phlebothromboses initiated by the intravenous injection of an endotoxin as reported in our previous studies.

Materials and Methods. The first study in rats (Table I) is the result of 2 different experiments. The exact number of animals actually utilized for these experiments is listed in Table I. A second study with 2 groups of animals was performed at a later date, comprising 9 control animals similar to those of group 3, and 10 treated for 4 weeks with triamcinolone. Results of this study are reported in Fig. 1.

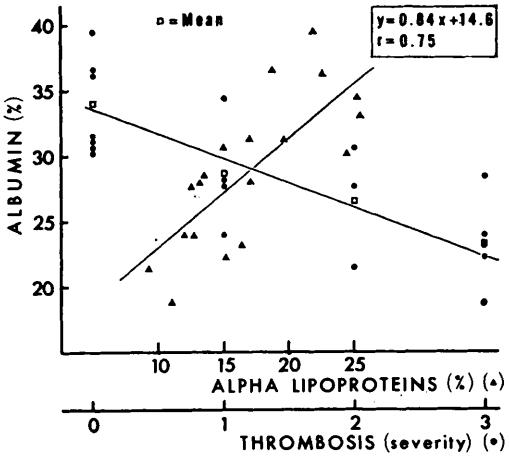

Fig. 1. Relationship between the serum α lipoproteins, the severity of thrombosis and the serum level of albumin in control and triamcinolone-treated rats (10 mg/kg of diet).

Holtzman male rats with an initial body weight of 140–170 gm were used in these experiments. The rats were housed 3–6/ cage, in a constant temperature environment and given, *ad libitum*, tap water and the following hyperlipemic diet: butter 38, casein 11, cellulose 15, cholesterol 5, salt mixture 4, sodium cholate 2, sucrose 23, and vitamin mixture 2 (weight %).[3] After 6 weeks of this dietary feeding, triamcinolone[4] was included in the diet of groups 2,4,5, and 6 (Table I),

* Supported by Grants from the Medical Research Council (MT-1444) and the Quebec Heart Foundation.

[1] Scholar of the Medical Research Council.

[2] Fellow of the Canadian Heart Foundation.

[3] The cellulose (alphacel), salt mixture (Wesson), vitamin mixture (vitamin diet fortification mixture), casein, cholesterol, and sodium cholate were purchased from Nutritional Biochemicals Co., Cleveland, Ohio.

[4] Triamcinolone: 9 α-fluoro-16 α-hydroxyprednisolone (Aristocort) kindly supplied by the Cyanamid Company of Canada through the courtesy of Dr. C. P. Gendron.

TABLE I. Thrombosis Prevention by Triamcinolone (Triam) Administration in the Diet, for 4 Weeks.

Triam included in the diet		none	15 mg/kg	none	15 mg/kg	15 mg/kg	20 mg/kg
Group[b]		1	2	3	4	5	6
No. of animals		22	18	30	9	16	12
Fasted (17 hours)		—	—	+	+	+	+
Replacement therapy (2 mg/kg)		—	—	—	—	+	+
Cholesterol[a] (mg/100 ml)		—	—	534 ± 41	392 ± 13	381 ±31	593 ± 68
Triglycerides[a] (mg/100 ml)		—	—	139 ± 12	132 ± 24	116 ± 9	176 ± 32
Lipoproteins[a] (%)	α	—	—	14 ± 0.9	15	23 ± 1.4	21 ± 1.4
	α_1	—	—	16 ± 1.0	16	17 ± 1.1	16 ± 1.0
	α_2	—	—	27 ± 1.1	25	25 ± 1.4	22 ± 1.2
	β	—	—	29 ± 1.2	30	24 ± 0.6	28 ± 1.6
	origin	—	—	14 ± 0.4	14	11 ± 0.6	13 ± 0.5
Plasma clotting time[a] (sec)		140 ± 9	205 ± 12	151 ± 5	143 ± 19	195 ± 7	238 ± 27
Body wt. gain[a] (gm)		—	—	77 ± 5	95 ± 8	98 ± 7	39 ± 4
Thrombosis: Incidence (%)		55	27	87	78	19	25
Severity (0–3)		1.1	0.4	2.4	2.1	0.2	0.5
Mortality (%)		83	27	87	78	6	9

[a] Mean ± SE. All the animals were fed for 10 weeks the following diet: butter 38, casein 11, cellulose 15, cholesterol 5, mineral salts 4, sodium cholate 2, sucrose 23, vitamins 2 (weight %). They were then injected with a *S. typhosa* lipopolysaccharide after the blood removal for the various determinations.

[b] Group comparison: Cholesterol: group 4 and 5 vs group 3, $p<0.001$; triglycerides: group 5 vs group 3, $p<0.2$; group 5 and 8 vs group 3, $p<0.3$. α lipoproteins: group 5 and 6 vs group 3, $p<0.001$; and plasma clotting time: group 2 vs group 1, $p<0.001$; group 5 and 6 vs group 3, $p<0.001$.

and in group 2 of study 2 (Fig. 1) by incorporation in melted butter, at the dosage indicated in the table (15–20 mg/kg of diet) or in the Fig. 1 (10 mg/kg). In addition, in groups 5 and 6 (Table I) and in group 2 (study 2), after 17 hours of fasting, triamcinolone (2 mg/kg of body wt.) was given as replacement therapy, by stomach tube, 17 hours and 2 hours before the endotoxin injection. At the end of the feeding period (10 weeks) in all animals, whether nonfasted (groups 1 and 2) or fasted (17 hours) (groups 3–6 and the 2 groups of study 2), approximately 3 ml of blood were removed in 2-ml siliconized syringes, using 20-gauge needles, from the jugular vein, under ether anesthesia, as reported in detail elsewhere (3). This was followed by an injection, through the same needle, of a *Salmonella typhosa* (0901, Boivin type) lipopolysaccharide (0.6 mg/kg) in saline. The plasma clotting time after recalcification in each rat was performed in duplicate in plastic tubes (cellulose nitrate, no. 654, or polycarbonate no. 2804, International Equipment Co., Boston, Massachusetts) as described previously (3). The lipemic parameters were evaluated on the sera of fasted animals only, and were determined individually for each rat except in group 4 where the lipoproteins were determined on the pooled sera of 2 animals. The total serum cholesterol was determined by a Technicon AutoAnalyzer, using the technique recommended by the manufacturer; the triglycerides, by the Van Handel method (5); the lipoproteins, by paper electrophoresis post-stained with fat-red 7B according to our modification (3) of the Straus and Wurm technique; and the proteins, also by paper electrophoresis stained with a bromophenol blue alcoholic solution. Following the endotoxin injection, a number of the animals died in 3–18 hours and the survivors were killed 24 hours after the injection. Autopsy was performed on all animals and the red hepatic infarcts were evaluated macroscopically and graded in terms of an arbitrary scale of 0–3. As reported in several of our previous studies, the red hepatic infarcts result from occlusive thrombosis of the hepatic veins.

Results. The intravenous injection of a *S.*

typhosa endotoxin in nonfasted rats fed the hyperlipemic diet for 10 weeks induced a 55% incidence of thrombosis with a severity of 1.1 (group 1, Table I). These thromboses, as already reported, occur mostly in the large hepatic veins and are responsible for huge and multiple red hepatic infarcts easily seen macroscopically. The incidence and the severity of thrombosis and the mortality rate were greatly reduced in the nonfasted rats receiving triamcinolone in the diet for 4 weeks prior to the endotoxin injection (group 2). The clotting time in this group was also much longer than in group 1.

In group 3, which was treated in exactly the same way as group 1 except that the animals were fasted for 17 hours before the endotoxin injection, the incidence and the severity of thrombosis were much higher than in the nonfasted group. Although we have not previously reported it, we have consistently observed this aggravation of thrombosis by fasting, under various experimental conditions. Except for the serum cholesterol values, the fasted rats receiving triamcinolone in the diet only (group 4) did not present any significant difference from their controls (group 3) as regards thrombosis, the clotting time, or the other lipemic parameters. In contrast to this, thrombosis was largely prevented and the clotting time markedly prolonged in groups 5 and 6, which, in addition to the hormone-feeding in the diet, received triamcinolone by stomach tube before the endotoxin injection as replacement therapy. The incidence and severity of thrombosis and the clotting time were comparable in these groups regardless of the dose of triamcinolone administered, and were also comparable to the hormone-fed, but nonfasted, group 2.

Similar changes were observed in groups 5 and 6 as regards the lipoproteins. In the triamcinolone-treated rats, there was a marked increase in the α lipoproteins (group 3). However, in the triamcinolone-treated animals not receiving replacement therapy (group 4), the proportion of the different fractions was the same as in the nontreated group 3.

The cholesterol and triglyceride levels were lower in groups 4 and 5, treated with the smallest dose of triamcinolone than in the nontreated group 3; but the figures were significant only for cholesterol. However, at the highest dose (group 6), the cholesterol and the triglycerides were more elevated than in the group 3, although not significantly so.

In Fig. 1, a correlation is seen between the percentage of albumin and that of α lipoproteins in the 9 animals nontreated and the 10 treated with triamcinolone. In addition, it appears there is also a relationship between this albumin level and the severity of thrombosis.

Discussion. Administration of triamcinolone for a 4-week period in the diet of hyperlipemic rats, at dosages little affecting the body weight, was nevertheless markedly effective in preventing thrombosis and improving the mortality rate following endotoxin injection. A higher dose that decreased the body weight did not appear to be more effective in this respect.

As in acute experiments, the protection afforded against thrombosis appears to be related to an increase in the α lipoproteins and in the clotting time. Although at first sight, these effects may appear to be similar to those of heparin, they do not seem to result from the release of heparin-like substances. Previous studies showed that such effects could not be blocked by protamine as could those of heparin (2) and, in addition, only after glucocorticoid therapy was the level of albumin increased (2). In the present experiment, a correlation was found between the level of albumin and that of the α lipoproteins. Although only a small number of animals was included in this part of the study, the results are probably significant, particularly since they confirm our earlier work (2). Some relationship between the severity of thrombosis and the level of albumin was also noted here. Since we have observed that addition of albumin to plasma *in vitro* (2) markedly prolongs the plasma clotting time, in confirmation of the *in vitro* work of other investigators (6), it seems that at least a part of the beneficial effects of glucocorticoids in the rats could be related to their effect on serum albumin, probably through an increase in albumin synthesis (7,8).

In our previous investigations, using pharmacologic doses of corticoids, the glucocorticoids were able to prevent thrombosis, despite the fact that they considerably increased the lipemia (1). In the rabbit (9,10) and in man (11), it has also been reported that high doses of glucocorticoids increase the serum cholesterol. In the present study, only the highest dose of triamcinolone increased the cholesterol and triglycerides in the serum. At low doses, a slight decrease of the lipemia was observed, as was previously noted in man when another glucocorticoid was used (12).

Although the beneficial effect of glucocorticoids in rats takes several days to appear (1), it can be lost very rapidly during the 17 hours of fasting or, at the most, over a 24-hour period, since rats do not eat much during the day. Because of this fact, replacement therapy, given by stomach tube, had to be instituted in order to determine the lipemic parameters in fasted rats. This shows the danger of interrupting corticoid therapy abruptly; the animals rapidly become as susceptible as the controls or even more so, as was suggested by preliminary experiments. This result seems to be interesting since it has been observed (13) that most cases of phlebothrombosis in corticoid or ACTH treated patients, occurred after withdrawal of the hormone.

Contradictory results have been reported in man concerning the effect of corticoids or ACTH on coagulation. One injection of cortisone (14) and prednisone (15) have been shown to shorten the clotting time. In this connection, it was only after several injections of a glucocorticoid or ACTH that we observed a prolongation of the clotting time in rat (1). In man, prolonged treatment with ACTH is followed, depending on the studies, by no significant alteration of the clotting time (16), by a decrease in the fibrinogen level (17), or by a decrease in the prothrombin level (13). A preliminary study in man performed in our Institute, has indicated that triamcinolone, administered once a day, at maintenance dosage (6–10 mg), for several weeks, could prolong the plasma clotting time and increase the percentage of α lipoproteins.

Summary. Administration of triamcinolone in the hyperlipemic diet of rats for a 4-week period, at dosages little affecting the body weight, afforded a marked protection against the large and multiple phlebothromboses initiated in these rats by the intravenous injection of a *S. typhosa* lipopolysaccharide. As in acute experiments, this protection could be related to a marked increase in the plasma clotting time, the percentage of α lipoproteins and of albumin. Independently of its protective effect on thrombosis, triamcinolone, at a low dosage, seemed to decrease the cholesterol levels, while it increased them at a higher dosage. However, there was no significant changes in the serum triglycerides.

The authors are particularly indebted to Dr. C. Allard and J. Champagne for some of the cholesterol and triglyceride determinations performed in this study.

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Received Dec. 20, 1967. P.S.E.B.M., 1968, Vol. 128.