breeding and the hatching of the young. In almost all cases, these appeared to result in increasing the responses again, which had been reduced to a lower level previously (figure 1). These phenomena can be explained by a change in response readiness which is to be considered as a result of changes in endogenous and exogenous stimuli[10]. From an ecological point of view, this is a highly adaptive mechanism: the animals increase their alarm behaviour, or readiness to give alarm calls, when critical stages in reproduction are reached, thus improving the survival chances of the young.

Another group of occurrences which can be correlated with a rise of the alarm response frequency is the appearance of natural enemies (figure 2). This can also be explained by an increase in response readiness and is a highly adaptive mechanism from an ecological point of view.

Following on from such an increase in response as a result of breeding biology or the appearance of an enemy, comes a rapid decrease and further habituation during the next few days. This raises the question as to whether the learning process continues from the level it had previously reached, or whether the learning process must start from the beginning again, e.g. the stored information has been eradicated by the newly received stimuli. The results indicate that the previously learned niveau is returned to after the appearance of an enemy. The question must remain open, however, in the case of changes observed as a result of breeding biology: is the disturbance one of long duration, and thus delays the onset of the old niveau, or does it make the stored information completely inaccessible so that the learning process must begin again?

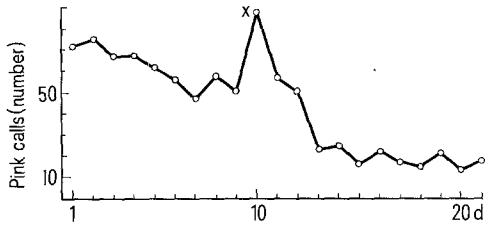


Fig. 2. Same as figure 1. The increase in response on day 10 is correlated with the previous appearance of a squirrel *(Sciurus vulgaris)* which remained near the nesting tree of the birds (×).

1 This investigation was supported by the Deutsche Forschungsgemeinschaft (Be 617/5).
2 I am indebted to Dr O. Anne E. Rasa for translating the manuscript into English, and to Dr H.-H. Bergmann for critical comments on an earlier draft of this article.
3 P. Marler, Ibis *98*, 231 (1956).
4 P. Marler, Behaviour Suppl. V (Leiden 1956).
5 W. Fleuster, J. Orn. *114*, 417 (1973).
6 H. Zucchi and H.-H. Bergmann, Experientia *31*, 817 (1975).
7 R.A. Hinde, Proc. R. Soc. Lond. *B 142*, 331 (1954).
8 R.F. Thompson and W.A. Spencer, Psychol. Rev. *73*, 16 (1966).
9 H.V.S. Peeke and M.J. Herz, in: Habituation, parts 1 and 2. Academic Press, New York, London 1973.
10 Ch. Becker-Carus, Z. Tierpsychol. *30*, 321 (1972).

Hageman factor activation and tight junction disruption in mice challenged with attenuated endotoxin[1]

R.I. Walker, M. Porvaznik, June E. Egan and A.M. Miller

Experimental Hematology Department, and Veterinary Medicine Department, Armed Forces Radiobiology Research Institute, Bethesda (Maryland 20014, USA), 12 June 1978

Summary. Endotoxin treated with chromium chloride is less toxic to mice than the parent molecule, but can disrupt intestinal permeability barriers and has an enhanced ability to activate Hageman factor.

Treatment of endotoxin with chromium chloride ($CrCl_3$) reduces its toxicity when injected i.p. into mice[2,3]. These endotoxin-chromium complexes are of interest because certain useful biological effects of the toxin may remain intact after treatment and because comparison of changes in host responses to treated and untreated endotoxin preparations may illuminate mechanisms responsible for pathologic effects of the toxin.

In this report we compared Hageman factor (HF) activation in mice challenged with untreated and $CrCl_3$-treated endotoxin. HF is a common initiator for kinin generation, intrinsic clotting, and plasma fibrinolytic systems[4]. Activation of HF apparently occurs in humans undergoing gram-negative shock[5] or following injection of volunteers with purified endotoxin[6].

We have recently associated disruption of tight junctional barriers between adjacent epithelial cells of the intestine with endotoxin-induced inflammation[7]. This phenomenon could lead to a self-sustaining endotoxemia[8] if host defenses against endotoxin from the intestine are impaired. We reported evidence for this event in mice made neutropenic by radiation prior to challenge with endotoxin attenuated with ferric chloride[9]. For these reasons, the effects of $CrCl_3$-treated endotoxin on tight junctional structure and survival of irradiated mice treated with oral antibiotics will be determined.

Male B6CBF1 mice were inoculated i.p. with a lethal dose of *Salmonella typhosa* lipopolysaccharide W (Difco). In some experiments, mice were decontaminated with oral bacitracin-neomycin mixtures as previously described[9]. Unirradiated mice were given 0.8 mg of the endotoxin, but mice irradiated 7 days previously with 1000 rads ^{60}Co (40 rad/min) were given only 0.3 mg of the toxin. Endotoxin was treated with chromium by adding 5 µl stock $CrCl_3$ (0.3 g/ml) to 1 ml of endotoxin (10 mg/ml pyrogen-free saline)[2]. This mixture was allowed to stand at 25 °C for 30 min and then was diluted 10 times with physiologic saline.

Blood for analysis was collected by retroorbital bleeding into plastic tubes containing 3% sodium citrate. Bleeding was initiated by insertion and immediate withdrawal of glass capillary tubes containing the anticoagulant. The citrated plasma prepared from this blood was assayed for HF activity using factor-deficient serum in a modification of the procedure of Hardisty and Macpherson[10]. Segments of ileum were removed from mice killed by cervical dislocation and fixed by immersion in cold 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.2). Samples

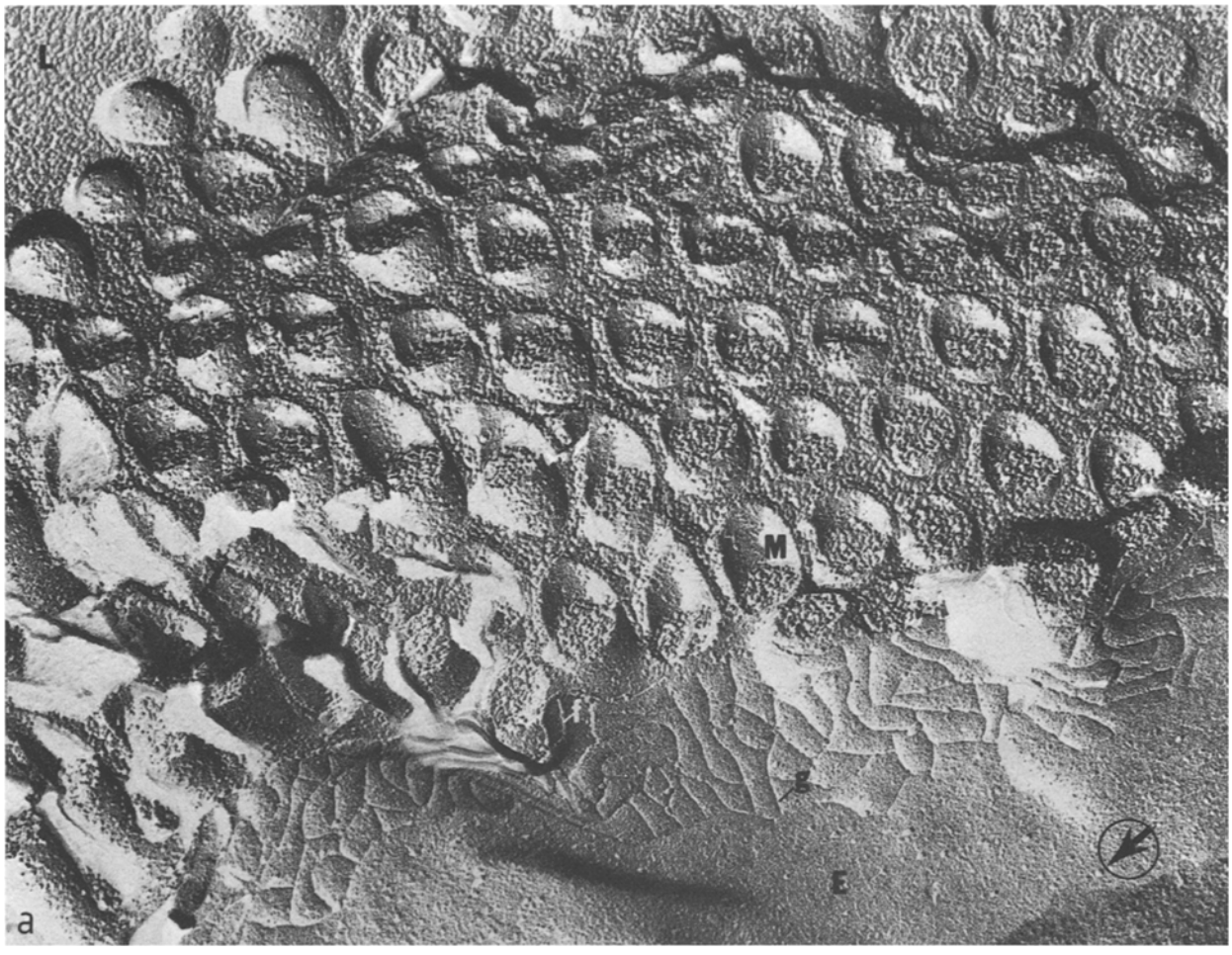


Table 1. Activation of Hageman factor* in mice challenged with normal and chromium-treated endotoxin

	Controls (15 min)		Untreated endotoxin		Chromium-ET	
	Saline	Chromium	15 min	5 h	15 min	5 h
Unirradiated***	61.8 ± 12.8	52.0 ± 6.8	61.6 ± 15.0	18.9 ± 8.6	32.8 ± 12.3**	21.2 ± 9.7
Irradiated***	44.7 ± 10.5	-	37.6 ± 15.7	-	23.6 ± 12.7**	-

* Expressed as mean ± SD % of activity obtained at time shown after challenge. Each sample consisted of blood pooled from 2 or 3 mice, and 5–18 samples comprised each group. ** Significantly deviated from saline control values ($p < 0.05$). *** Unirradiated mice were challenged with 0.8 mg *S. typhosa* endotoxin, but irradiated mice only received 0.3 mg of the toxin.

Table 2. Percent survival of irradiated and unirradiated mice challenged with normal or chromium-treated endotoxin

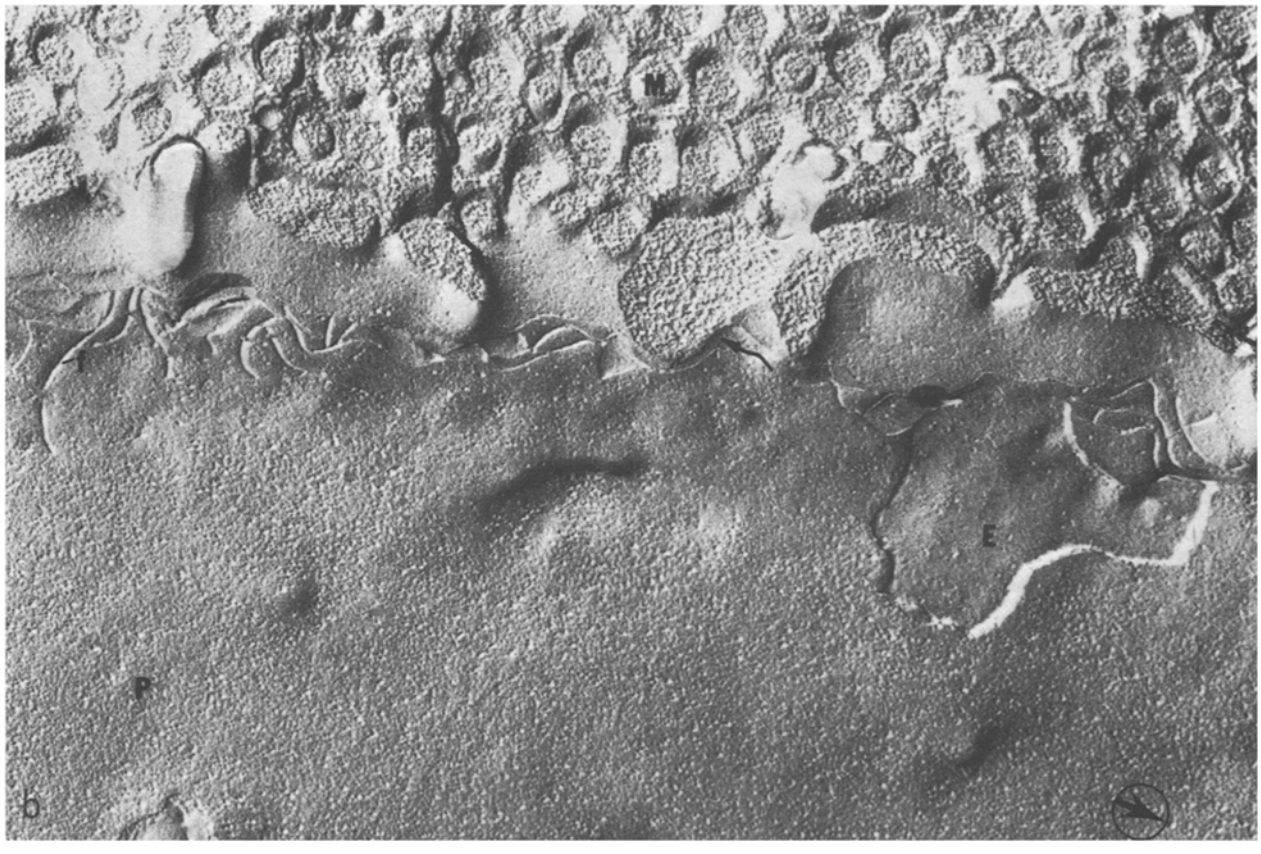
	Unirradiated		Irradiated*			
	Conventional ET	CrET	Conventional ET	CrET	CrET Conventional	Decontamination
Dead (total)	10/10	0/20	16/24	13/23	11/14	4/14
Percent mortality	100	0	66	57	79	29

* Mice were irradiated 7 days previously with 1000 rads ^{60}Co.

were freeze-fractured and examined with electron microscopy as previously described[7].

Neither chromium alone nor untreated endotoxin altered HF activity within 15 min after injection into unirradiated mice (table 1). Endotoxin treated with $CrCl_3$, however, reduced HF levels approximately 50% within this time period. Similar differences in activation of HF were seen between groups of irradiated mice injected with treated or untreated endotoxin. At 5 h after inoculation of mice with endotoxin, HF levels were depressed equally in animals receiving either treated or untreated endotoxin. The somewhat lower levels of HF seen in irradiated mice are consistent with findings of decreased HF activity in dogs 3 days after 400 rads ^{60}Co[11].

Survivals of irradiated and unirradiated mice challenged with the 2 endotoxin preparations were compared (table 2).



a This micrograph demonstrates a continuous, uninterrupted tight junctional complex observed between epithelial cells in the ileum from mice 7 days after receiving 1000 rad of γ-radiation and injected with saline as a control. The arrows point to the tight junction structure, fibrils (f) and membrane E-fracture face (E) grooves (g). The encircled arrow indicates the direction of platinum shadowing during the replication process. Intestinal lumen (L), apical microvilli (M). $\times$ 71,670; scale bar = 0.5 µm. *b* This micrograph demonstrates the kind of disrupted tight junctional complexes observed in ilea from mice challenged with $CrCl_3$-treated endotoxin 7 days after 1000 rad of γ-radiation. Notice that the concertina-like meshwork of structural fibrils (*f*) attenuate and actually become discontinuous (arrow) at 1 point and may permit the bypass diffusion of substances (e.g. endogenous endotoxin) from the lumen into the body cavity. Membrane P-fracture face (P). $\times$ 59,400.

Treatment with chromium completely obviated the mortality associated with 0.8 mg of endotoxin for unirradiated mice. In contrast, mortality was similar in irradiated mice challenged with 0.3 mg of either treated or untreated endotoxin. Decontamination of the intestinal tract of irradiated mice significantly reduced the mortality associated with these toxin preparations.

Tight junctional barriers between adjacent epithelial cells of the intestine of irradiated mice were examined. All 56 tight junctional complexes examined from mice injected with saline 7 days after irradiation were found intact (figure a) and looked like normal tight junctions from unirradiated control preparations. In contrast (figure b), some junctional complexes were observed to be disrupted in preparations from unirradiated (1/26 junctional interfaces examined) and irradiated (4/42 junctional interfaces examined) mice challenged with $CrCl_3$-treated endotoxin.

Accelerated activation of HF obtained with $CrCl_3$-treated endotoxin may contribute, at least in unirradiated animals, to attenuation of toxicity. One mechanism for this effect could be reduction by activated HF of platelet aggregation[12] induced by endotoxin. In irradiated mice, any benefits derived from a similarly more efficient mobilization of the inflammatory response to endotoxin could be offset by loss of other components of the response. For example, leakage of unattenuated endotoxin from the intestine may be initiated in response to inflammation induced by $CrCl_3$-treated endotoxin. We have previously demonstrated the importance of peritoneal leukocyte mobilization in protection against endotoxin entering the systemic circulation via the peritoneal cavity[7,13]. The absence of these cells in irradiated animals could account for deaths associated with challenge with $CrCl_3$-treated endotoxin. This concept is supported by our survival data in decontaminated mice challenged with $CrCl_3$-treated endotoxin and also previously with $FeCl_3$-treated endotoxin[9,13], plus the tight junction disruption we observed in irradiated mice challenged with $CrCl_3$-treated endotoxin. In addition, $CrCl_3$-treated endotoxin may cause a transient increase in susceptibility to infection[14] which we have previously shown occurs at this time after irradiation[15].

Although endotoxin can complex directly with purified HF[16,17], we suspect that endotoxin-induced activation in vivo is a mediated effect. This could account for the greater than 15-min delay for HF activation following challenge with untreated endotoxin. Mediators such as epinephrine[18] and granulocyte-released substances[19] are known to activate HF.

1 We wish to thank Drs M.W. Brightman and T.S. Reese from the Department of Neurocytology, National Institutes of Health for the use of their Balzers freeze-fracture instrument.

2 S.J. Prigal, A. Herp and J. Gerstein, J. Reticuloend. Soc. *14*, 250 (1973).

3 S.L. Snyder, R.I. Walker, T.J. McVittie and J.M. Sheil, Can. J. Microbiol., in press (1978).
4 O.D. Ratnoff, in: Immunobiology, p. 135. Ed. R.A. Good and D.W. Fisher. Sinauer Associates Inc., Stanford 1971.
5 J.W. Mason, U. Kleeberg, P. Dolan and R.W. Colman, Ann. intern. Med. *73*, 545 (1970).
6 H.R. Kimball, K.L. Melmon and S.M. Wolff, Proc. Soc. exp. Biol. Med. *139*, 1078 (1972).
7 R.I. Walker and M. Porvaznik, Infect. Immun., in press (1978).
8 D.T. Caridis, M. Ishiyama, P.W.H. Woodruff and J. Fine, J. Reticuloend. Soc. *14*, 513 (1973).
9 C.B. Galley, R.I. Walker, G.D. Ledney and M.R. Gambrill, Exptl Hemat. *3*, 13 (1975).
10 R.M. Hardisty and J.C. Macpherson, Thromb. Diath. haemorrh. *7*, 215 (1962).
11 P. Klir, J. Pospisil, E. Skala, Z. Dienstbier and O. Tacheci, Strahlentherapie *146*, 713 (1973).
12 J. Aznar, J. Mayans and J.A. Aznar, Haemostasis *3*, 20 (1974).
13 R.I. Walker, S.L. Snyder, P.Z. Sobocinski, K.F. McCarthy and J.E. Egan, Can. J. Microbiol., in press (1978).
14 E. Suter, in: Bacterial Endotoxin. Ed. M. Landy and W. Braun. Institute of Microbiology, New Brunswick, NJ, 1964.
15 R.I. Walker, G.D. Ledney and C.B. Galley, Radiat. Res. *62*, 242 (1975).
16 D.C. Morrison and C. Cochrane, J. exp. Med. *140*, 797 (1974).
17 S.D. Revak and C.G. Cochrane, Thromb. Haemostasis *3*, 520 (1976).
18 D.M. Zubairov and L.G. Popova, Thromb. Res. *8*, 587 (1976).
19 R.G. Lerner, S.I. Rapaport and J.M. Spitzer, Thromb. Diath. haemorrh. *20*, 430 (1968).

Independent response of 2 characters to selection for insensitivity to photoperiod in *Pyrrhocoris apterus*

A. Honěk[1]

Research Institutes for Plant Production, Institute of Plant Protection, Ruzyně 507, 161 06 Praha 6 (Czechoslovakia), 22 September 1978

Summary. The obligatorily macropterous strain of *Pyrrhocoris apterus* has been selected for 29 generations. Nevertheless, the strain retained a photoperiodically controlled diapause.

In *Pyrrhocoris apterus* L. (Heteroptera, Pyrrhocoridae), 2 characters are controlled by photoperiod: the diapause and the wing polymorphism[2-4]. Under constant short-day conditions, this species diapause and virtually all individuals become brachypters with reduced membrane of the fore-wing. Under constant long-day, the insects do not diapause and a fraction of population becomes macropterous with fully developed membranes of the fore-wing. In our cultures we selected *Pyrrhocoris* for macropterousness for 29 generations. The insects were bred by standard methods: held in groups in plastic vials and supplied with linden seed and water. The photoperiod used was either light 18: dark 6 (long day) or light 12: dark 12 (short day) and temperature 26 ± 1 °C.

In the 1st–22th generation, the selection was applied in long-day conditions by taking groups of 25–50 macropterous individuals of the previous generation as parents for the next generation. By these means the proportion of macropters in the population increased to about 70% within 9 generations and remained approximately constant, with smaller variations caused presumably by minor variations in laboratory conditions. In short-day conditions, the percentage of macropters remained fairly low till 23rd generation, when a small fraction of macropters appeared. Then the new method of selection was adopted: the macropters from short-day were activated by transfer to long-day conditions and used to establish further generation. By this procedure, the percentage of macropters increased within a few generations, both in long-day (to more than 90%) and short-day (to about 70%) conditions. Thus a large fraction of this selected population can be considered as photoperiodically insensitive with respect to wing polymorphism (figure 1).

The diapause properties were tested in this selected material in 26th and 28th generations. In contrast to the lack of morphoregulative effect of photoperiod, the selected macropterous strain, like the wild material, retained full capacity for diapause induction. Under constant short-day, both brachypters and macropters did not exhibit any sign of reproductive activity for at least

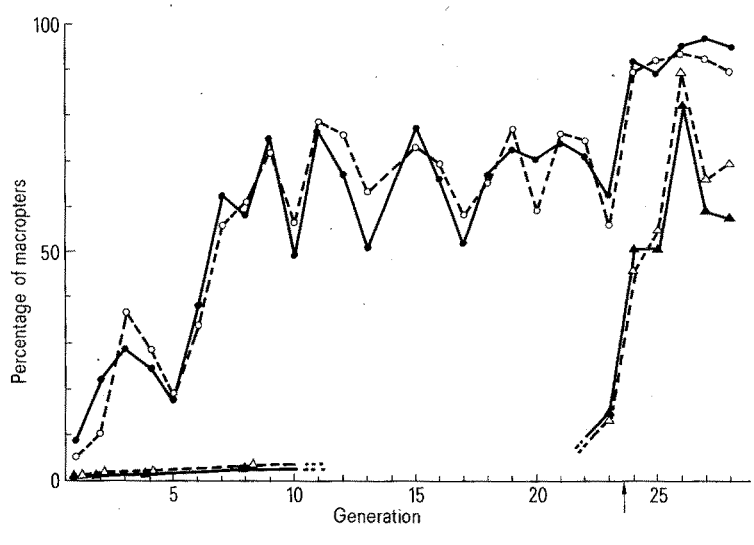


Fig. 1. The percentage of long-winged individuals in the strain selected for macropterism during 29 generations. ●——● long-day, males; O----O long-day, females; ▲——▲ short-day, males; △----△ short-day, females; ↑ new method of selection adopted (see explanation in text).