blood glucose must be maintained for a certain period before its reduction to stimulate HGH secretion.

Our observation is difficult to interpret, but raises some questions on the regulatory manner of HGH secretion mediated by glucose metabolism. If the stimulation of HGH secretion occurs by intracellular glucose deprivation(1-3) in the hypothalamus(2,7) as proposed, glucose infused for a short period in most cases is unable to induce enough metabolic shift in the hypothalamic cells to stimulate HGH secretion, in spite of other definite responses such as plasma NEFA change. An alternative explanation may be that the direct stimulant of HGH secretion is not the extracellular or intracellular glucose itself, but the metabolites or other substances related to glucose metabolism, which are different quantitatively or qualitatively in the conditions of short and long continuous glucose infusion. Since plasma NEFA change was similar in all cases, it is apparent that there is no correlation between the rise of plasma HGH and the value of plasma NEFA. In these respects, our results may support the observation of Quabbe *et al*(8), who showed the absence of correlation between plasma HGH rise and blood glucose or plasma NEFA level during a 24-hour fast in normal subjects.

Summary. Serial plasma HGH concentrations were measured, in 18 normal subjects, following continuous intravenous glucose infusion performed for periods of 3, 10, 20 and 30 minutes. In 5 of 8 cases infused for 3 minutes, no rise of plasma HGH was observed in spite of the rapid fall of blood glucose with (4 cases) or without (1 case) its reduction below the fasting level. The 3 other cases infused for 3 minutes and all 10 cases of 10, 20 and 30 minutes' infusion showed definite rise of plasma HGH at 90-180 minutes following the infusion. From the results obtained, it appears that the falling blood glucose *per se* is not a direct stimulus, and the reduction of blood glucose below the fasting level is not necessarily a stimulus to HGH secretion.

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Role of Protein Component of Endotoxin in Modification of Host Reactivity.* (32346)

HENRY H. FREEDMAN, ALFRED E. FOX, R. SUZANNE WILLIS, AND BENJAMIN S. SCHWARTZ

Department of Microbiology, Warner-Lambert Research Institute, Morris Plains, N. J.

The bacterial endotoxins are characterized as lipopolysaccharides(1,2). Boivin-type preparations of endotoxins contain significant amounts of protein which may be largely removed by phenol-water partition(3) yielding purified and potent Westphal-type endotoxins of low protein nitrogen content. Ribi and co-workers(4) have succeeded in preparing a potent protein-free endotoxin having the usual range of toxic and protective properties and containing only 0.2% nitrogen, accounted for by hexose-amine content. This lack of dependence of biological activity upon protein content or presence has led to

* Present address: Waldemar Medical Research Foundation, Woodbury, N. Y.

the current widespread belief that the protein component associated with endotoxic activity in the native state is superfluous (1,2,5,6). While this view is demonstrably correct in normally reactive adult animals (7), its pertinence to the problem of the origin of host reactivity to endotoxins(8,9) has not been established. Specifically, it has been emphasized(10,11) that many aspects of host reactivity to endotoxins closely resemble delayed hypersensitivity reactions. The identification of antigenicity of the bacterial endotoxins with the polysaccharide components of the molecule has made difficult (10) the reconciliation of adult host reactivity with acquired delayed hypersensitivity, since there is no clear evidence that purely polysaccharide antigens as a class can give rise to the delayed hypersensitivity states commonly achieved with protein antigens(12, 13). The present study arose from our recent observations(14) that the protein-free endotoxin differed from several Boivin or aqueous-ether endotoxins in modification of host reactivity to subsequent endotoxin challenge.

Materials and methods. Endotoxin preparations of *Salmonella enteritidis*(4), kindly provided by Dr. Edgar Ribi, included: an aqueous-ether extract (Se 289/293), 3.44% N; a protein-rich side fraction (293A), 4.76% N; a 0.2 mole fraction ethanol precipitate (293B), 1.88%N; a refined protein-free preparation, as aluminum-citrate complex (Se 289/293T), 0.21% N. Boivin and Westphal type endotoxins of *Salmonella abortus equi* (Difco) were also employed. Female CD-1 mice, weighing 18-20 g, were given 0.01 μg *i.p.* of an endotoxin preparation and were subsequently tested with 1 or 10 μg *i.p.* of the same or a different endotoxin. Elevation of numbers of hemolysin-forming spleen cells 48 hours after the test dose of endotoxin(15) was determined by localized hemolysis in agar, as previously described(14). Inhibition of water intake after endotoxin(16) was measured during the 16 hours following the test dose of endotoxin as previously described (17). Female albino rabbits, weighing 1½-2 kg, were injected with 0.01 μg *i.p.* of an endotoxin and were subsequently skin-tested with 1 and 0.1 μg *i.d.* of the same or another endotoxin. Delayed inflammatory reactions were graded at 24 and 48 hours after *i.d.* injection. Responses to challenge of pretreated animals were compared in each assay with responses of non-pretreated controls given the same test dose. Individual assays in mice employed control and experimental groups of 5 mice each, and experiments were repeated several times. Where appropriate, mean ± standard error was computed for each group and significance of difference between means accepted for "p" values less than 0.001. Dilutions for injection were made in non-pyrogenic saline and rigorous precautions to avoid contamination with extraneous endotoxins were observed.

TABLE I. Influence of Pretreatment with 0.01 μg of Aqueous-Ether (Se 289/293) or Protein-Free (Se 289/293T) *S. enteritidis* Endotoxin on Inhibition of Water Intake Produced in Mice by 1 μg Test Dose of Either Endotoxin Given 10 Days Later.

Endotoxins		Water intake, ml/5 mice/16 hr		
		Experiment		
Pretreatment	Test	A	B	C
Untreated controls		29	28	30
——	Aqueous-ether	16	17	16
——	Protein-free	20	21	16
Aqueous-ether	Aqueous-ether	10	9	12
"	Protein-free	11	10	7
Protein-free	Aqueous-ether	16	15	17
"	Protein-free	20	18	18

Results. Both the aqueous-ether endotoxin and the protein-free endotoxin of *S. enteritidis* inhibit water intake in normal mice at a dose of 1 μg (Table I). These data, from 3 separate experiments, demonstrate further that mice given 0.01 μg of the aqueous-ether endotoxin 10 days earlier respond to challenge with greater than usual inhibition of water intake, whether the test endotoxin is the aqueous-ether or the protein-free preparation. In contrast, pretreatment with the protein-free endotoxin does not modify reactivity to either endotoxin given 10 days later.

The data in Table II demonstrate that a 10 μg dose of either the aqueous-ether or the protein-free endotoxin causes a marked increase in the numbers of spleen cells producing hemolysins for sheep red blood cells,

TABLE II. Influence of Pretreatment with 0.01 μg of Aqueous-Ether (Se 289/293) or Protein-Free (Se 289/293T) *S. enteritidis* Endotoxin on Increase in Numbers of Hemolysin-Forming Spleen Cells 48 Hours After 10 μg Test Dose of Either Endotoxin Given 10 Days Later.

Endotoxins		No. hemolysin-forming cells per 1/5 spleen, mean ± S.E.
Pretreatment	Test	
Untreated controls		2 ± .7
——	Aqueous-ether	45 ± .9
——	Protein-free	32 ± 2.6
Aqueous-ether	Aqueous-ether	142 ± 5.4
"	Protein-free	128 ± 5.6
Protein-free	Aqueous-ether	43 ± 2.6
"	Protein-free	34 ± 1.7

found 48 hours after injection of normal mice. However, only pretreatment with the aqueous-ether preparation results in significantly greater than usual responses to either endotoxin given 10 days later, pretreatment with the protein-free preparation having no influence on subsequent reactivity. The failure of the 0.01 μg pretreatment doses alone to modify the numbers of hemolysin-forming spleen cells found at the time of assay has been documented(14). Additional experiments, using intervals of 1, 8, 12, 20, and 26 days between pretreatment and test dose, confirm that the protein-free endotoxin does not modify reactivity to a subsequent dose whereas the aqueous-ether endotoxin modifies reactivity biphasically(14). A comparison of the aqueous-ether, the protein-rich side fraction, and the 0.2 mole fraction ethanol precipitate preparations of *S. enteritidis* is given in Table III. Either of the first two causes a significant increase in numbers of hemolysin-forming spleen cells but the 0.2 mole fraction preparation is practically without effect. Again, the aqueous-ether endotoxin produces a significantly greater than usual increase in numbers of antibody-producing spleen cells in mice which had received 0.01 μg of this same endotoxin 10 days earlier. Mice given 0.01 μg of the protein-rich side fraction, however, are refractory to the test dose of the same endotoxin given 10 days later; whether a long-lasting endotoxin tolerance(14) is associated with the high nitrogen content of this preparation is being studied. Results obtained from studies with the Boivin and low-nitrogen Westphal types of endotoxins of *S. abortus equi* are given in Table IV. Once again,

TABLE III. Influence of Pretreatment with 0.01 μg of Aqueous-Ether (Se 289/293), Protein-Rich Side Fraction (293A) or 0.2 Mole Fraction Ethanol Precipitate (293B) Preparation of *S. enteritidis* on Increase in Numbers of Hemolysin-Forming Spleen Cells 48 Hours After 10 μg Test Dose of the Same Preparation Given 10 Days Later.

Endotoxins		No. hemolysin-forming cells per 1/5 spleen, mean ± S.E.
Pretreatment	Test	
Untreated controls		2 ± 1.3
——	Aqueous-ether	52 ± 2.4
Aqueous-ether	"	161 ± 5.6
——	Protein-rich	29 ± 1.7
Protein-rich	"	3 ± .4
——	0.2 mole fraction	6 ± 1.0
0.2 mole fraction	"	2 ± .9

TABLE IV. Influence of Pretreatment with 0.01 μg of Boivin or Westphal Type Endotoxin of *S. abortus equi* on Increase in Numbers of Hemolysin-Forming Spleen Cells 48 Hours After 10 μg Test Dose of Either Endotoxin Given 10 Days Later.

Endotoxins		No. hemolysin-forming cells per 1/5 spleen, mean ± S.E.
Pretreatment	Test	
Untreated controls		1 ± .9
——	Boivin	38 ± 2.6
——	Westphal	35 ± 2.2
Boivin	Boivin	104 ± 4.9
"	Westphal	127 ± 8.1
Westphal	Boivin	50 ± 2.9
"	Westphal	43 ± 2.7

both endotoxins elevate the numbers of hemolysin-forming spleen cells found 48 hours after injection. Here too, however, pretreatment with the Boivin endotoxin results in significantly greater than usual increases in response to either endotoxin given 10 days later, whereas pretreatment with the partially deproteinized Westphal endotoxin does not significantly alter subsequent reactivity to either endotoxin.

The influence of pretreatment of rabbits with the various endotoxin preparations of *S. enteritidis* and *S. abortus equi* on subsequent delayed skin reactivity to *i.d.* endotoxins is described in Table V. Only occasional normal rabbits suffered a 1+ reaction

TABLE V. Influence of Previous Endotoxin Treatment on Delayed Inflammatory Reactions Produced in Rabbits by Intradermal Injection 10 Days Later, Using Endotoxins of Varying Nitrogen Content.

Test endotoxins,* dose, μg i.d.	Delayed inflammatory reaction, at 48 hr				
	Pretreatment endotoxins,* 0.01 μg i.p.				
	None	Se 289/293	Se 289/293T	Sae B	Sae W
Se 289/293, 1.0	+, 0, +	3+, 3+, 2+, 2+	+, 0, +, +		
" , 0.1	0, 0, 0	+, +, 0, +	0, 0, 0, 0		
Se 289/293T, 1.0	0, 0, 0	+, 3+, 0, +	0, 0, 0, 0		
" , 0.1	0, 0, 0	0, +, 0, 0	0, 0, 0, 0		
Sae B, 1.0	0, +, +			3+, 3+	0, 0
" , 0.1	0, 0, 0			+, +	0, 0
Sae W, 1.0	0, 0, +			3+, 3+	+, +
" , 0.1	0, 0, 0			2+, 2+	0, +

* Endotoxins: Se 289/293, aqueous-ether *S. enteritidis*; Se 289/293T, protein-free *S. enteritidis*; Sae B, Boivin *S. abortus equi*; Sae W, Westphal *S. abortus equi*.

to a 1.0 μg dose of any endotoxin, and as expected no Arthus-type reactivity was seen with the low doses used. For the two *S. enteritidis* endotoxins, pretreatment with 0.01 μg of the aqueous ether preparation results in enhanced skin reactivity to either endotoxin injected 10 days later but pretreatment with the protein-free preparation is without effect. Thus the distinction between these two endotoxin preparations in their influence upon subsequent host reactivity is found in all 3 experimental models studied. For the two *S. abortus equi* endotoxins, pretreatment with the Boivin preparation results in increased skin reactivity on subsequent test with either endotoxin whereas pretreatment with the Westphal-type preparation fails to significantly alter normal reactivity to either, consistent with the data in Table IV.

Discussion. The present data extend the applicability of our previous findings(14) on modification of host reactivity to subsequent endotoxin challenge to the inhibition of water intake caused by endotoxins(16) and to delayed inflammatory reactions produced by intradermal injection of endotoxins(11) in rabbits. Taken together with our recent observations(18) on sensitization to the ordinarily inactive Boivin endotoxin of *Brucella abortus*, these data clearly indicate that host reactivity to endotoxins is conditioned by previous exposure. In particular, modification of host reactivity expressed as increased reactivity to subsequent challenge appears to require a protein component present in the Boivin or aqueous-ether preparations of the organisms studied. That loss of capacity to induce hyper-reactivity occurs before total removal of protein from lipopolysaccharide is demonstrated by the findings with the low-nitrogen Westphal preparation.

If normal adult host reactivity to bacterial endotoxins has an origin in acquired delayed hypersensitivity the presence of a protein or peptide determinant in the endotoxin macromolecule would be expected, as has been suggested(9). The studies reported here provide a basis for believing that such a mechanism in fact exists. The protein associated closely with endotoxic activity in the native state is present during natural exposure of the host to relevant bacteria. It is of interest that Watson and Kim(9) have postulated for the endotoxin molecule the presence of a widely distributed "related antigen" to which delayed hypersensitivity develops resulting in the "secondary toxicity" of their scheme. The protein function described here and previously(14) may be considered a demonstration of their proposed "related antigen." These data do not contribute to the question of a "primary toxicity" for endotoxins; from our present(18) and continuing studies with *B. abortus* endotoxin, however, a "primary toxicity" does not appear to be obligatory. The activity in normal or hyperreactive animals of these low-nitrogen endotoxin preparations which cannot themselves induce hyperreactivity remains to be explained. It would appear unlikely that Ribi's deproteinized endotoxin contains a significant, but unrecognized, contiguous portion of the ori-

ginal protein. Further studies on the protein moiety, and its possible role in endotoxin tolerance, are in progress. That the *in vivo* biological properties of endotoxins may be mediated by distinctive antigenic properties of the intact macromolecule(19) must be considered.

Summary. The influence of endotoxins of varying nitrogen content on water intake and numbers of hemolysin-forming spleen cells in mice and on delayed skin reactivity in rabbits has been investigated. Pretreatment with an aqueous-ether endotoxin of *S. enteritidis* results in heightened reactivity to a subsequent test dose of the aqueous-ether or protein-free (Ribi) endotoxin, whereas pretreatment with the protein-free endotoxin fails to modify subsequent reactivity to either endotoxin. Similarly, pretreatment with the Boivin endotoxin of *S. abortus equi* increases reactivity on subsequent test with the Boivin or Westphal endotoxin, whereas pretreatment with the partially deproteinized Westphal endotoxin does not enhance reactivity to subsequent test with either endotoxin. The implications of these findings for the hypothesis that adult host reactivity to endotoxins is contributed to by an acquired delayed hypersensitivity are discussed.

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Some Factors Affecting the Interferon-Induced Antiviral State. (32347)

SAMUEL BARON, CHARLES E. BUCKLER, HILTON B. LEVY AND ROBERT M. FRIEDMAN

Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, and National Cancer Institute, National Institutes of Health, Bethesda, Md.

The interferon system may be composed of 2 distinct cellular proteins. The first is the interferon protein which is induced by viral infection(1) and which has been interpreted to react with cells to induce a second substance—the proposed intracellular, antiviral protein(2). The present communication will consider some of the factors which influence the interferon-induced, second component of the interferon system—the antiviral protein.

Materials and methods. Interferon was produced in the serum of NIH strain mice by intravenous injection of Newcastle disease virus, as previously described(3). It was