biphasic cortical response, as well as 8 well defined and regular after-potentials are clearly visualized. Noise and transient discharges have virtually disappeared.

Where applicable, PET offers certain advantages over computer averaging techniques. PET is not limited by channel capacity. Resolution is restricted only by the width of the oscilloscope trace or the slow variability of the waveform. PET will recognize 2 or more waveforms which alternate, rather than display a spurious composite. PET reproduces median or modal waveform rather than mean waveform. Whereas computer procedures store and process both information and noise, PET retains only repeated information and eliminates noise. In addition, line width and line brightness may be employed as indices of waveform reliability. Brighter and more narrow portions of a multiphasic wave will indicate greater repeatability or reliability of that waveform component. However, the determination of the variability in a biological potential must be statistically evalulated on the basis of individual waveform analysis(2).

This technique has been successfully employed in investigation of electroretinogram components as well as the postnatal development of evoked cortical potentials(3). With the addition of suitable electronic triggering circuitry, PET may be employed with aperiodic spontaneous electrical activity.

1. Dawson, G. D., *EEG & Clin. Neurophysiol.*, 1954, v1, 65.

2. Collins, R. L., Meckler, R. J., Ordy, J. M., Samorajski, *Anat. Rec.*, 1964, v148, 272.

3. Ordy, J. M., Samorajski, T., Collins, R. L., Nagy, A., in preparation.

Received September 2, 1964. P.S.E.B.M., 1964, v117.

Hyperfibrinogenemia and Thrombocytopenia After Staphylococcal Enterotoxin.* (29680)

H. SUGIYAMA, E. M. MCKISSIC, JR. AND T. HAYAMA†

Food Research Institute and Department of Microbiology, University of Chicago, Chicago, Ill.

Staphylococcal enterotoxin elicits several biological responses resembling those produced by bacterial endotoxin. The first experimental feeding of enterotoxin to monkeys results in a transitory refractoriness to the emetic stimulus of enterotoxin which is distinct from permanent immunity. The temporal sequence of this tolerant state is similar to that of the "non-specific" resistance to infections and endotoxins following a single dose of endotoxin(1). Intradermal injection of staphylococcal enterotoxin alters the dermal reactivity of rabbits and guinea pigs so that injection of epinephrine into the same site results in a local hemorrhagic response. The local Shwartzman phenomenon can be produced by staphylococcal enterotoxin if bacterial endotoxin is employed as the provoking agent(2). The intravenous injection of staphylococcal enterotoxin into cats results in a biphasic hyperthermia(3). A reticuloendothelial blockading agent (Thorotrast) sensitizes monkeys to the emetic action of intragastrically administered enterotoxin(4). Mice and rabbits pretreated parenterally with staphylococcal enterotoxin are more sensitive to the lethal action of bacterial endotoxins (5).

The present communication is concerned with the effect of staphylococcal enterotoxin on 3 other changes effected in rabbits by bacterial endotoxins. Fibrinogen levels gradually increase after a single intravenous injection of bacterial endotoxin while the blood platelet counts decrease(6,7). Administration of bacterial endotoxin induces the appearance of an altered form of fibrinogen

* Supported by grant from U. S. Public Health Service, EF-77.

† Permanent address: Inst. of Food Microbiology, Chiba Univ., Japan.

which precipitates with heparin in the cold (8).

Materials and methods. Two Enterotoxin B preparations of about 95% purity were used. Prep I was obtained by the previously described method(9) while II was purified by substitution of carboxymethylcellulose ion exchange step for starch electrophoresis and alcohol precipitation. The intragastric (i.g.) emetic potency of Prep I has gradually decreased so that at the time of the present experiments 30 μg/monkey was needed to provoke vomiting in 50% of previously unused monkeys. Prep II had i.g. emetic ED_{50} of 10 μg/animal. The intravenous (i.v.) emetic ED_{50} of these samples for monkeys was about 0.8 and 0.5 μg/kg body weight for Prep I and II, respectively.

Prep III was Enterotoxin A sample in which at least 50% of the organic material was enterotoxin. The i.g. emetic ED_{50} for monkeys was between 70-100 μg per animal. Boiling the toxin sample for 30 minutes reduced its lethal activity for rabbits so that i.v. injections could be used(5).

Bacterial endotoxin was lipopolysaccharide (LPS) of *Escherichia coli* 0111:B4 (Difco Laboratories) dissolved in pyrogen-free saline by holding in 80°C water bath for 15 minutes.

New Zealand male rabbits of 2-3 kg were used after conditioning in the laboratory for at least 7 days. Blood was obtained by cardiac puncture; injections were into the ear vein. Rhesus monkeys (*Macaca mulatta*) were challenged with enterotoxin either i.v. or i.g.; blood was taken from the saphenous vein.

Fibrinogen was assayed as either heat precipitable fibrinogen of Stirland's method(10) or as clottable fibrinogen by a modification of the method of Ware *et al*(11). Enumeration of blood platelets was by the direct counting technic(10) using Rees-Ecker diluent. Blood serotonin was determined by spectrophotofluorometry of protein-free filtrate of whole blood obtained by zinc sulfate precipitation (12). The effect of enterotoxin on these parameters was calculated as the percentage of control values (immediately before challenge) at selected intervals after enterotoxin injection. Heparin-precipitable fibrinogen was determined by holding heparinized plasma (1 mg/ml of whole blood) for 2 hr in an ice-water bath(13).

Results. The average increases of fibrinogen titers of rabbits challenged intravenously with several doses of 3 enterotoxic preparations are shown in Table I. The results with Prep I showed gradual rises in titer attaining a maximum around 24 hr post challenge when the fibrinogen concentration was 2 to nearly 3 times the control values with the higher toxin dosages. Individual rabbits showed increases of up to 3.8 times pre-injection levels, while an occasional rabbit was much less sensitive. The titer at 48 hours was generally slightly lower than at 24 hours.

The hyperfibrinogenemia was not due to hemoconcentration. Two rabbits showed fibrinogen concentrations 1.9 and 3.4 times greater than pre-injection levels 24 hours after injection of 50 μg/kg of Prep II. The erythrocyte counts showed no significant changes (5.5 and 5.3 $\times$ 10^6 pre-injection to 5.6 and 5.2 $\times$ $10^6/mm^3$ 24 hours after enterotoxin).

The present data with staphylococcal enterotoxin are similar to those reported after a single injection of bacterial endotoxin except for the continuing increase in fibrinogen over a 48-hour sampling period after endotoxin(6). This suggests a difference in the response of the rabbit to these 2 toxins.

TABLE I. Average Increases of Rabbit Fibrinogen Titers (Percentage of Pre-injection Level) After Intravenous Injections of Staphylococcal Enterotoxin.

Challenge toxin, μg/kg		No. animals	Hr after enterotoxin 4	18	24	30	48
Saline		6	105	104	112	115	118
Prep I	50	4	154	185	280	251	210
	10	4	161	170	258	228	230
	1	4	—	—	185	—	187
Prep II	10	3	—	—	242	—	213
	2	3	—	—	260	—	247
	.5	3	—	—	150	—	—
Prep III	25	2	—	197	203	—	190
	10	2	—	—	231	—	177
	1	2	—	—	200	—	205

Prep I and II: Enterotoxin B; Prep III: heated Enterotoxin A.

The effect on plasma fibrinogen concentration of monkeys challenged with enterotoxin by different routes was studied. Essentially the same elevation as in rabbits occurred in 2 monkeys given intravenous threshold emetic doses of Prep I. The fibrinogen titers were 2.5 and 2.6 times greater than the pre-injection levels 24 hours after enterotoxin. Intragastric administration of enterotoxin near the minimal emetic dose produced no significant rise of plasma fibrinogens in 6 monkeys; 3 of these animals vomited to enterotoxin challenge. However, when large doses (50 and 70 times the estimated minimal emetic dose) were fed, the titers were 3.2 and 2.4 times higher than control values.

Heparinized plasma obtained from rabbits injected 2, 4 and 6 hours previously with enterotoxin did not precipitate when held in an ice-water bath for 2 hours. Two rabbits were tested at each time interval with doses of 100 and 50 μg/kg for the 2 Enterotoxin B preparations and 50 and 25 μg/kg for the heated Enterotoxin A. Maximum quantity of heparin-precipitable fibrinogen occurs 2 to 4 hours after injection of endotoxin(8). The plasma of rabbits administered *E. coli* LPS gave positive reactions at 3 hours post-challenge with 5 μg (3 of 3) and occasionally at 1 μg/kg (1 of 4). Of the total of 6 rabbits tested with the higher dosages of each of the 3 enterotoxic preparations, one plasma sample gave a strong and another a doubtful reaction when the plasma was obtained 24 hr after enterotoxin challenge.

A fall in the 5-hydroxytryptamine concentration of blood was initially observed in rabbits given intravenous injections of highly purified staphylococcal enterotoxin. Since the serotonin of blood is carried by the blood platelets(14), it was not surprising to find that the depression of serotonin concentration corresponded to a fall in blood platelet counts. Representative results with Prep I are presented in Table II.

Heated Prep III produced similar effects. The initial precipitous decrease in circulating platelets after bacterial endotoxin(6,7) was not observed with staphylococcal enterotoxin. The maximum thrombocytopenia occurred around 24 hours post-enterotoxin. No indication has been obtained that enterotoxin released serotonin from rabbit platelets *in vitro*.

TABLE II. Thrombocytopenia and Decreased Blood Serotonin (Percentage of Pre-injection Level) After Intravenous Injections of Enterotoxin Prep I.

Enterotoxin, μg/kg		Hr after enterotoxin 4	18	24	30	48
Saline	Platelet	97	98	95	103	92
	Serotonin	102	97	98	98	97
10	Platelet	85	61	37	34	48
	Serotonin	71	59	42	39	59
1	Platelet	79	69	58	65	77
	Serotonin	82	64	64	70	83
.5	Platelet	—	67	57	61	85
	Serotonin	93	70	62	69	98

Although not obvious from the Tables, the degree of hyperfibrinogenemia and thrombocytopenia may not be dose related. The magnitude of the changes was not necessarily greater with the larger doses of a given enterotoxin preparation when a certain dosage was exceeded.

Discussion. Hyperfibrinogenemia and thrombocytopenia induced in rabbits are further similarities in the biological responses evoked by both staphylococcal enterotoxin and bacterial endotoxin. Although these 2 responses elicited by the 2 toxins are superficially alike, the time sequences are sufficiently dissimilar to indicate that they result from the action of 2 different agents. Moreover, enterotoxin does not stimulate the appearance of the heparin-precipitable fibrinogen at the time optimum for demonstration of this phenomenon with endotoxin.

The small dosage of essentially pure enterotoxins that is effective is good evidence for the enterotoxin basis of the present observations. The good correlation between the relative potencies of these and similar enterotoxin preparations as emetic agents (i.g. route) for monkeys and as agents sensitizing mice to LPS(5) supports this conclusion.

The activity of the heated Prep III in these experiments is greater than would be surmised from the i.g. emetic potency for monkeys of the unheated enterotoxin. Limited

experience suggests that heating of this particular enterotoxin sample increases its activity for the parenteral route.

Hyperfibrinogenemia is an indication of either increased fibrinogen synthesis by the liver, decreased removal from the circulation, or both. The possible role of the clearance mechanism in the present observation is suggested by the increase in sensitivity of monkeys to i.g. administered enterotoxin after reticuloendothelial system blockade(4). On the other hand, the increased plasma fibrinogen may represent a more non-specific response of the animal to the stress of the toxic actions of enterotoxin. Hyperfibrinogenemia occurs in animals and man subjected to many conditions of stress(11).

Increased utilization or removal of blood platelets from the circulation related to vascular damage(15) may play a role in the shock syndrome observed after i.v. injections of large doses of staphylococcal enterotoxin.

The renal cortical necrosis of the generalized Shwartzman phenomenon is a rare occurrence using enterotoxin as both the sensitizing and provocative doses. Failure of staphylococcal enterotoxin to stimulate the appearance of the heparin-precipitable material at optimum time for demonstration of this response with bacterial endotoxin may be the explanation. An important role in this phenomenon has been ascribed to this altered fibrinogen(16).

Summary. Parenteral injection of staphylococcal enterotoxin increased plasma fibrinogen levels of rabbits and monkeys. The maximum titer of between 2 to 3 times the prechallenge concentration was attained in 24 hours after enterotoxin administration. Enterotoxin also depressed the blood serotonin concentration of rabbits. This drop in blood serotonin corresponded to the decrease in number of circulating blood platelets. Both antigenic types of enterotoxin tested induced hyperfibrinogenemia and thrombocytopenia. The dosages required to induce a significant change in these two responses was close to the minimal emetic dose for monkeys by the intravenous route.

1. Sugiyama, H., Bergdoll, M. S., Dack, G. M., *J. Infect. Dis.*, 1962, v111, 233.

2. Bokkenheuser, V., Cardella, M. A., Gorzynski, E., Wright, G. G., Neter, E., PROC. SOC. EXP. BIOL. AND MED., 1963, v112, 18.

3. Clark, W. G., Borison, H. L., *J. Pharmacol. Exp. Therap.*, 1963, v142, 237.

4. Sugiyama, H., McKissic, E. M., Bergdoll, M. S., PROC. SOC. EXP. BIOL. AND MED., 1963, v113, 468.

5. Sugiyama, H., McKissic, E. M., Bergdoll, M. S., Heller, B., *J. Infect. Dis.*, 1964, v114, 111.

6. McKay, D. G., Shapiro, S. S., *J. Exp. Med.*, 1958, v107, 353.

7. Davis, R. B., Meeker, W. R., Bailey, W. L., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 774.

8. Thomas, L., Smith, R. T., von Korff, R., *ibid.*, 1954, v86, 810.

9. Bergdoll, M. S., Sugiyama, H., Dack, G. M., *Arch. Biochem. Biophys.*, 1959, v85, 62.

10. Lynch, M. J., Raphael, S. S., Mellor, L. D., Spare, P. D., Hills, P., Inwood, M. J. H., *Medical Laboratory Technology*, W. B. Saunders Co., Philadelphia, 1963.

11. McKenzie, J. M., Celander, D. R., Guest, M. M., *Am. J. Physiol.*, 1963, v204, 42.

12. Udenfriend, S., Weissbach, H., Brodie, B. B., in *Methods of Biochemical Analysis*, 1963, v6, Interscience Publishers, New York.

13. Lee, L., *J. Exp. Med.*, 1963, v117, 366.

14. Humphrey, J. H., Jacques, R., *J. Physiol.*, 1954, v124, 305.

15. Roy, A. J., Djerassi, S., Neitlich, H., Farber, S., *Am. J. Physiol.*, 1962, v203, 296.

16. Lee, L., *J. Exp. Med.*, 1962, v115, 1065.

Received September 2, 1964. P.S.E.B.M., 1964, v117.