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Clinical Chemistry of Staphylococcal Enterotoxin Poisoning in Monkeys

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ABSTRACT

CRAWLEY, GERALD J. (Fort Detrick, Frederick, Md.), JOHN N. BLACK, IRVING GRAY, AND JACK W. BLANCHARD. Clinical chemistry of staphylococcal enterotoxin poisoning in monkeys. Appl. Microbiol. **14**:445–450. 1966.—Clinical chemistry values were examined in 90 monkeys administered a purified preparation of staphylococcal enterotoxin, type B, intravenously. These studies showed an early release of epinephrine accompanied by a mild increase in blood glucose. This was followed by progressively developing prolonged hypoglycemia. An early increase in blood-urea nitrogen occurred, presumably as a result of both prerenal azotemia and functional renal failure seen in association with the observed hypotension. Serum protein, Ca, and Cl concentrations decreased with time. Pi levels increased, whereas Na and K concentrations in serum remained unchanged. Serum enzyme concentrations were unchanged, with the exception of serum glutamic oxaloacetic transaminase, which rose rapidly when compared with prechallenge control observations or with values from sham-challenged monkeys. These changes were statistically significant. These results suggested that enterotoxin administered intravenously produced early change in glucose metabolism, possibly related initially to catecholamine release and later to increased utilization of glucose and metabolic acidosis. Other findings were compatible with tissue breakdown at as yet undetermined locations and with loss of endothelial membrane integrity, as evidenced by loss of protein from the vascular space.

Most information relative to biochemical changes in animals and man due to staphylococcal enterotoxin has been obtained from experiments involving oral administration of impure toxin preparations or ingestion of contaminated food. Rozhkova (19) stated that hypoglycemia, hypoprothrombinemia, cholesterolemia, and uremia developed in enterotoxin food poisoning. Mikhlin et al. (18) demonstrated increased enterokinase activity in the stools of patients suffering from staphylococcal food poisoning. Sugiyama, Bergdoll, and Dack (24) reported that serum glutamic oxaloacetic transaminase (SGOT) increased after feeding enterotoxin to monkeys; they attributed this to tissue damage. Staphylococcal enterotoxin did not alter the activity of serum phosphatase or cholinesterase, apyrase, tissue monamine oxidase, cholinesterase, dehydrogenase, acetylase, or enzyme systems that regulate oxidative phosphorylation (Sugiyama, *unpublished data*), and no effect on blood glucose, serum sodium, or potassium was observed in monkeys. In another study, Sugiyama (*unpublished data*) reported that the intravenous administration of purified enterotoxin to rabbits was followed by a decrease in the blood serotonin levels which correlated with a decrease in circulating platelets.

Previously we reported data concerning the binding, tissue distribution, and excretion of a purified preparation of staphylococcal enterotoxin, Type B (SEB), isotopically labeled with I^{131}, as well as data on body fluid compartment changes in monkeys after intravenous administration (4; Crawley et al., J. Infect. Diseases, *in press*). In subsequent investigations described herein, biochemical changes were observed after intravenous administration of this purified toxin to rhesus monkeys.

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MATERIALS AND METHODS

A purified preparation of SEB (20) was administered intravenously (iv) in a dose of 25 μg/kg (unless otherwise noted) to young adult *Macaca mulatta* monkeys, weighing 2.3 to 3.5 kg. After prolonged conditioning to insure an optimal state of health, the monkeys were allowed to accustom themselves to specially designed metabolic chairs for several days before challenge. To facilitate repeated blood sampling, the posterior vena cava was cannulated 2 to 4 days prior to study. The animals were maintained on a commercial monkey biscuit diet.

Blood glucose values were obtained by the method of Folin and Wu (9) on a group of 35 monkeys. Blood-urea nitrogen (BUN) content was determined in 32 animals by direct nesslerization (11). A direct biuret method was used for the assay of total protein in 34 monkeys (15). The concentration of serum Na, K, Pi, and Cl was determined by automated analysis (14, 17, 27), whereas Ca levels were assayed with an ultramicrotechnique (6). Serum glutamic pyruvic transaminase (SGPT), SGOT, lactic dehydrogenase (LDH), and aldolase content were determined by the Sigma methods (2, 21, 22). Ceruloplasmin was assayed by the method of Houchin (13). Blood samples were collected 2 or 3 days prior to challenge to serve as control values. For assay of Na, K, Cl, Pi, and Ca, samples were obtained at 1, 3, 5, 8, 12, 24, and 30 hr after challenge. Six monkeys served as sham-challenge controls.

A group of eight fasting monkeys was used to examine blood glucose concentrations in a serial fashion for 3 hr after challenge. Fasting control samples were collected on three successive mornings. On the 3rd day, six monkeys were challenged with SEB, and two served as fasting sham-challenged controls.

The catecholamine response to enterotoxin was studied in six animals. To avoid variations due to excitement, these animals were held in an isolated area for 1 week prior to study. Only one individual entered the area during the course of the experiment for the purpose of care, blood sampling, and administration of the SEB. These animals were sham-bled three times daily for 3 days to accustom them to manipulation of the cannula. On day 4, precontrol blood samples were obtained. The monkeys were again sham-bled on days 5 and 6, and were challenged with 20 μg/kg the next day. Blood samples were taken at 1, 3, 6, and 24 hr postchallenge. For analysis, the method of Weil-Malherbe and Bone (26), as modified by Gray and Young (12), was used.

Data were statistically analyzed by means of the Student *t* test (23) for differences from prechallenge control values, sham-bled controls, or both.

RESULTS

After iv SEB, a slight but significant increase in blood glucose occurred in the fasting monkeys (Table 1) which became maximal at 60 min. Blood glucose then began to fall and was significantly depressed below control by 180 min. In the nonfasting monkeys, relative hypoglycemia varied in onset and duration. It was observed in all animals studied, and group averages were significantly ($P < 0.001$) below control values at 6, 24, and 48 hr. Sham-challenged fasted controls also showed a lowered glucose concentration with increased length of fasting, but hypoglycemia never approached the magnitude observed after SEB.

TABLE 1. *Blood glucose concentrations after SEB challenge*

Time	Blood glucose (mg/100 ml ± 1 SD)	
	Fasting (6 monkeys)	Nonfasting (35 monkeys)
hr		
Prechallenge	76 ± 12	95 ± 20
0.25	73 ± 10	
0.5	79 ± 10	
0.75	85 ± 14	
1	92 ± 12	
2	68 ± 8	
3	57 ± 7	
6		*81 ± 23** (34)
24		*67 ± 21* (27)
48		*74 ± 15* (15)

* Italics indicate values significantly different from prechallenge $P = < 0.001$. Number of surviving animals is indicated in parentheses.

A significant elevation of BUN (Table 2) was evident at 3 hr; maximal values were recorded generally at 24 hr. A persistent elevation of the group average remained at 48 hr, although many animals had returned to normal by then.

There was a gradual, progressive, and statistically significant decrease in plasma protein concentration during the course of intoxication (Table 2). Ceruloplasmin values indicated a trend toward elevation which reached a significant difference from control by 48 hr.

A significant elevation in SGOT values was present by 3 hr and persisted through 20 hr. The SGOT level remained slightly elevated at 48 hr, although the difference from prechallenge values at that time was no longer significant.

SGPT control value was 16 ± 10 units; a maximum of 27 ± 19 was reached at 6 hr, but was not significantly different. Similarly, LDH and aldolase were not significantly different from prechallenge levels of 834 ± 175 and 22 ± 8 units, respectively.

The results of serum electrolyte studies are indicated in Table 3. Na and K varied randomly in different individuals with no definite trend or pattern. Serum Na values varied between 142 and 143 meq/liter regardless of time. Similarly, K

TABLE 2. *Clinical chemistry values after SEB challenge*

Time	BUN	Protein	SGOT	Ceruloplasmin
hr	*mg/100 ml*	*g/100 ml*	*units*	*units*
Prechallenge	19 ± 6 (32)*	7.0 ± 1.0 (34)	32 ± 13 (14)	70 ± 5 (6)
3	*28 ± 3*† (9)		*70 ± 38* (9)	
6	*30 ± 11* (31)	*6.2 ± 0.8* (31)	*77 ± 30* (12)	76 ± 6 (5)
20			*57 ± 24* (8)	78 ± 9 (5)
24	*52 ± 23* (27)	*6.1 ± 0.7* (30)		
48	*38 ± 23* (24)	*5.7 ± 0.9* (20)	38 ± 27 (6)	*82 ± 8* (5)

* Number of monkeys.
† Italics indicate values significantly different from prechallenge $P = < 0.001$.

TABLE 3. *Serum electrolyte values of SEB- and sham-challenged monkeys*

Type of challenge	Amt (meq/liter ± 1 SD)			
	Time	Cl	PO_4	Ca
	hr			
SEB	Prechallenge	108 ± 11 (18)*	4.2 ± 2.1 (19)	3.9 ± 0.8 (36)
	1	107 ± 11 (16)	4.5 ± 1.9 (17)	
	3	106 ± 14 (19)	5.1 ± 3.0 (19)	
	5	106 ± 11 (18)	5.6 ± 2.4 (18)	3.6 ± 0.6 (29)
	8	106 ± 11 (18)	5.4 ± 2.0 (14)	
	12	101 ± 12 (15)	*6.8 ± 3.7* (16)	
	24	*95 ± 7*† (14)	*8.2 ± 3.4* (16)	*3.3 ± 0.7* (23)
	30	*95 ± 12* (15)	*7.0 ± 2.8* (15)	
	48			*3.4 ± 0.5* (18)
Sham	Prechallenge	111 ± 13 (5)	3.7 ± 2.0 (5)	
	1	105 ± 9 (5)	3.1 ± 1.4 (6)	
	3	105 ± 4 (6)	3.6 ± 2.4 (6)	
	5	103 ± 8 (6)	3.1 ± 1.4 (6)	
	8	105 ± 5 (5)	3.5 ± 0.9 (6)	
	12	100 ± 6 (6)	3.6 ± 0.6 (6)	
	24	101 ± 8 (5)	4.1 ± 2.0 (5)	
	30	108 ± 5 (4)	3.2 ± 1.0 (5)	

* Number of monkeys.
† Italics indicate values significantly different from prechallenge $P = < 0.001$.

levels varied between 3.7 and 4.1 meq/liter. Serum Cl levels showed an initial decline at 12 hr, which decreased to significantly low values at 24 and 30 hr. These were also significantly lower than sham-challenged control values at 30 hr. There was a progressive elevation in Pi which reached a maximum at 24 hr. Group averages differed significantly from prechallenge control values by the 12th hr. The difference from sham-challenged control values was significant as early as 5 hr, an indication that the observed increase was not due to a diurnal change. Serum Ca levels also decreased and were significantly below prechallenge control values at 24 and 48 hr.

Mean catecholamine responses are shown in Fig. 1. A rise in serum epinephrine content occurred in all monkeys studied. The actual response time seemed to be within the first 3 hr, with average values returning almost to normal by 6 hr. All values were in the normal range by 24 hr. The norepinephrine concentration showed no significant change from prechallenge values at the times of sampling.

Approximately 60% of the monkeys died at the dose administered. Death occurred from 6 hr to 9 days; the majority, within 3 days, and the remainder, during days 6 to 9. Emesis with or without diarrhea occurred frequently but they were not consistent symptoms, even in animals with lethal intoxication.

DISCUSSION

After iv administration, purified staphylococcal enterotoxin causes vomition, diarrhea, anorexia,

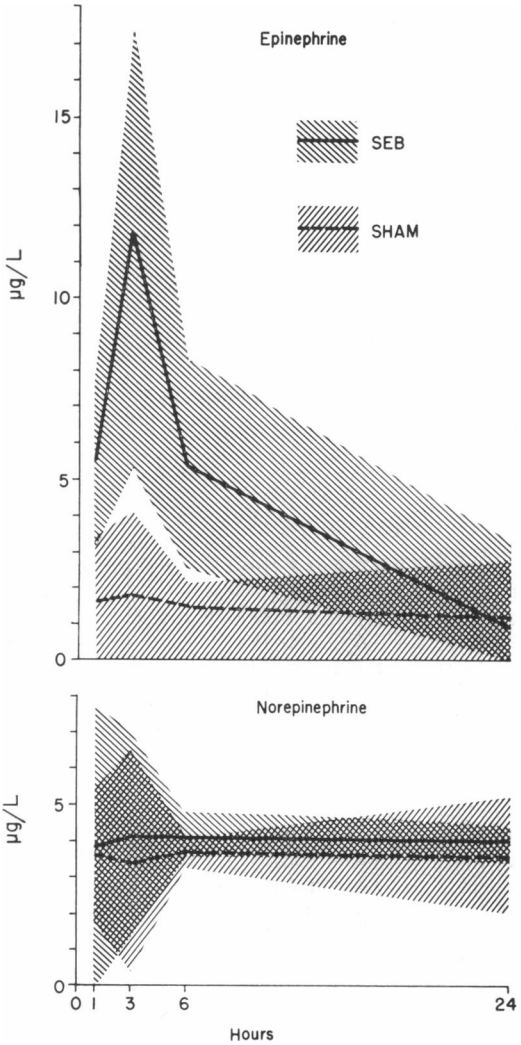

FIG. 1. *Catecholamine responses in six monkeys first studied in sham fashion and then after SEB given 3 days later. Data shown represent means ± SE. Elevations of epinephrine 1, 3, and 6 hr after SEB were statistically significant.*

fever, profound shock with oliguria or anuria, dyspnea, mental depression, and terminal cyanosis. There occurs an initial transient leukopenia followed by severe leukocytosis associated with a large number of immature leukocytes (4). Although the cause of death following SEB remains indefinite, a fall in intravascular fluid volume has been noted with an increase in lung weights (Crawley et al., J. Infect. Diseases, *in press*.) There is an apparent causal relationship between the central nervous system and the emetic response to oral toxin (1; Sugiyama, *unpublished data*; 25).

Although early studies attempted to define the manner in which staphylococcal enterotoxin induced emesis, it was observed that preparations of staphylococcal enterotoxin of increasing purity generally retained emetic potency when administered orally, but produced fewer additional side manifestations. The highly potent SEB used by us retained emetic potency on oral administration, but often produced death after iv administration without antecedent emesis or diarrhea. Death often failed to occur until long after the anticipated time of vomition (1 to 4 hr). This observation suggested the possibility that the mechanisms leading to death after iv SEB intoxication differed from those postulated mechanisms leading to vomition.

The various alterations in serum or blood chemistry noted herein undoubtedly represented the summated influence of many physiological, metabolic, and endocrine factors of divergent nature. None of these changes could be correlated with lethality or death, although the timing and sequential nature of the changes suggested that they undoubtedly were related to the intoxication.

The sequential changes in blood catecholamine levels and in blood glucose were similar to the well-documented changes observed after the administration to animals of bacterial lipopolysaccharides (3, 5, 7, 8, 16; Sanford et al., J. Clin. Invest. **38**:1039, 1959). As with endotoxin, iv SEB administration was rapidly followed by increased serum epinephrine levels. This, in turn, may have contributed to the elevation of blood glucose levels. The late hypoglycemia observed after endotoxin administration appeared to be the result of depletion of hepatic and muscle glycogen stores; a similar sequence could explain the late and prolonged relative hypoglycemia noted after SEB. Similar patterns of carbohydrate response have recently been observed after anthrax toxin administration to experimental animals (Klein et al., J. Infect. Diseases, *in press*). Although the magnitude and duration of hypoglycemia were subject to individual variation, it appeared that animals which regained their appetite recovered more rapidly from the hypoglycemia; however, such animals did not always survive the challenge. Other factors which may have contributed to the prolonged hypoglycemia included severe anorexia, increased metabolic utilization of glucose in the peripheral tissues, and increased serum lactate concentration.

Although a depression of urinary output, occasionally reaching complete anuria, accompanied the development of hypotension after SEB administration, there appeared to be no change in renal histology (D. Yost, *personal communication*). Despite absence of renal structural change,

an increase in BUN was evident within 3 hr and continued to rise for 24 hr. The degree of increase in BUN did not correlate with mortality. The observations of anuria or oliguria and BUN elevation in the absence of structural renal change were compatible with functionally impaired renal clearance. In addition, the magnitude of the rise in BUN over a 3-hr period suggested that approximately twice the normal amount of urea nitrogen had been produced within the body, assuming that a 2.5-kg monkey normally excreted about 125 mg of urea every 3 hr (W. R. Beisel, *personal communication*) and that the distribution of urea was equal throughout total body water. Such rapid accumulation of urea, greater than that which could be accounted for by renal failure alone, was suggestive of prerenal azotemia, possibly the result of increased catabolism of protein or gluconeogenesis.

An explanation for the decrease in serum proteins is not readily apparent. The decrease may be due to loss of proteins by increased degradation, loss through blood sampling, loss into extravascular spaces, to dilution of the protein by an increase in vascular fluids, or to any combination of these factors. Although blood lost through sampling and flushing of the venous catheter with small amounts of saline may have contributed to the observed fall in serum proteins, such was not the case in control animals similarly bled. Furthermore, no samples of blood were collected between 24 and 48 hr, yet a considerable fall in serum protein concentration occurred then. These observations are compatible with the postulate that leakage of serum proteins from the vascular space (Crawley et al., J. Infect. Diseases, *in press*) was a factor in the observed fall in their concentration.

Of the enzymes studied in a serial fashion, only SGOT became elevated. High tissue concentrations of SGOT normally exist in liver, kidney, and skeletal and cardiac muscle of man, and may be released after injury to any of these tissues, although serum levels do not usually become elevated for 6 to 8 hr. None of these tissues revealed cellular damage on histological examination (D. Yost, *personal communication*). The observed increase in SGOT was present in many monkeys within 3 hr of SEB administration. Since the value of other serum enzymes which primarily reflect liver damage remained within normal limits, it is probably permissible to assume that the liver was not the source of the rise in SGOT.

The similarity of timing in the rise of serum Pi with that of the BUN permitted oliguria to be an attractive explanation. Similarly, the late fall in serum Ca may be related to functional renal failure and acidosis. Elevation of Pi and depression of Ca has also been noted after endotoxin administration (5, Peterson and Brunson, Federation Proc. **18**:499, 1959), although the magnitude of Ca changes was related to severity of symptoms after bacterial endotoxin administration (10).

Serum Na and K concentrations remained essentially stable throughout the observed toxemia. The amount of saline administered in flushing cannulae was inconsequential. In contrast, the slight but significant late fall in Cl remains unexplained. Loss due to vomition or in occasional diarrhea was far too small to explain the observed changes and should have produced changes at an earlier time, inasmuch as the diarrhea or vomiting, if present, ended within the first 3 to 4 hr. A relationship of this change to acidosis, with a "chloride shift" into red blood cells, may be a more likely possibility.

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