Discussion. The present investigation has revealed that partially and highly purified preparations of staphylococcal enterotoxin alter the dermal reactivity of rabbits and guinea pigs to epinephrine. The question arises whether the epinephrine effect is due to enterotoxin itself or to some other component present in the purified preparations. If the epinephrine effect were due to a hitherto unrecognized component of purified enterotoxin, it would have to be present in all preparations examined thus far, and, to account for the high activity, in high concentration. On the other hand, if the epinephrine effect is in fact due to enterotoxin, a new basis will be provided for biological assay of this elusive toxin, and inferences may be drawn regarding its mode of action.

The altered reactivity to epinephrine induced by the enterotoxin preparations in rabbits resembles that produced by staphylococcal extracts, by viable or killed staphylococci, and by endotoxins from gram-negative bacteria. Although similarities are evident in the biological activities of enterotoxin and endotoxin, differences are also apparent. Thus it would appear that although enterotoxin is active as a preparatory dose in the Shwartzman reaction, it differs from endotoxin in being inactive as a provoking dose. The available chemical and physical data also reveal no similarities between classical endotoxin and the staphylococcal enterotoxin, and accordingly it is suggested that the latter product should not be referred to as endotoxin.

Summary. Intravenous injection of 6 preparations of the S-6 type of staphylococcal enterotoxin in rabbits resulted in the appearance of dermal lesions at the site of subsequent intradermal injections of epinephrine. Intradermal injection of staphylococcal enterotoxin followed by administration of epinephrine into the same sites also resulted in the appearance of dermal lesions in both rabbits and guinea pigs. Enterotoxin was effective in amounts as small as 0.0001 μg. The toxic factor was not destroyed by heating for 15 min at 60°C or 100°C. Antiserum failed to neutralize the toxic effect.

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Induced Drinking in Dogs: Comparative Effects of Hypertonic Sodium Chloride and Sorbitol. (27938)

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Work on the hypothalamus of various mammals(1-5) indicates the existence of an area whose stimulation leads to drinking, which is responsive to osmotic stimuli, and whose destruction profoundly inhibits water intake. Substances which remain osmotically

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TABLE I. Comparison of Responses to 10% NaCl and 57.4% Sorbitol.

Dog No.	1		2		3		4		5		6	
Solution inj	NaCl	Sorb.	NaCl	Sorb.	NaCl	Sorb.	NaCl	Sorb.	NaCl	Sorb.	NaCl	Sorb.
ml of water drunk/ml	10.80	9.3	3.4	3.1	4.3	3.2	6.2	1.8	5.5	n.t.	n.t.	5.0
of solution inj.	10.75	10.1	3.3	3.0	7.1	5.4	2.75	1.7	7.4	n.t.	n.t.	3.8
Each value is avg	9.50	12.9	5.3	3.4	8.4	3.0	5.9	1.2	5.6	n.t.	n.t.	5.4
of 4 to 6 consecutive	9.33	9.7	4.0		8.67	5.3	5.67			n.t.	n.t.	4.4
hourly responses	11.0											
Over-all averages	10.3	10.5	4.0	3.2	7.1	4.2	5.1	1.6	6.2	n.t.	n.t.	4.7

n.t. = not tested.

active in the body fluids produce roughly comparable drinking responses when injected in hypertonic solutions(6). A quantitative examination of the stimulus-response relationship when 20% NaCl is injected was performed using a more satisfactory technic(7, 8). If hypertonicity of the extracellular fluid provides the stimulus to "thirst" receptors, it should be possible to demonstrate quantitative equivalence in the effects of equal numbers of particles of any 2 inert, impermeable substances. In the present experiments, the effects of sodium chloride and sorbitol were reinvestigated using the more precise technic (7) allowing measurements of latencies and close control over the conditions of stimulation.

Method. Male mongrel dogs, fasted for 24 hours before each experiment but having had free access to water, were prepared for injection by a method previously described which allows repeated injections without handling or restraint of the animal(7). A polyethylene tube was inserted into the jugular vein and the dog left undisturbed for one to 2 hours in a metabolism cage before a series of 4 to 6 successive hourly injections of NaCl or sorbitol was begun. Each dose was 10 ml of either 10% NaCl or its osmotic equivalent, 57.4% sorbitol. The latency and amount of every draught was recorded, including the drinking through 2 hours after the last injection. The total water taken was divided by the total volume injected in each series to arrive at the average drinking response.

Results. The range of individual average drinking responses was from 4.0 to 10.3 ml of water drunk for each ml of 10% NaCl injected and 1.6 to 10.5 ml of water per ml of 57.4% sorbitol (Table I). The consistency of each animal's drinking indicates that the procedure successfully controls, within limits, the many unknown factors which could act as adequate stimuli to drinking, or facilitate its induction. However, little consistency either in amount or latency was observed in successive injections during an experiment (Fig. 1, Table II). Some odd individual response patterns were noted (Fig. 1 A,B). Drinking following each injection was most commonly done at one draught, but it was sometimes distributed over several draughts, often far apart, during the hour following. Refractoriness to one stimulus was often followed by a greater volume drunk after the next injection.

Equivalence between the 2 substances was observed in only one of the animals (Dog No. 1, Table I, Fig. 1 C). The varying degrees of decreased sorbitol effectiveness in the other dogs appear to parallel their over-all latency type (Table II); in general, the longer the dog waited before responding, the less effective was a sorbitol stimulus. However, latency itself did not correlate with amount of drinking (Tables I and II). Dog

TABLE II. Relationship Between Latency Patterns and Sorbitol Effectiveness.

Dog	NaCl/ Sorb.*	Total responses	% responses† (min.) 0-3	3-10	10-60	% no response
1	.98	48	81	4	6	9
2	1.25	32	28	28	24	20
3	1.7	38	35	19	27	19
4	3.2	39	15	10	30	45

* Ratio is quotient of over-all averages from Table I.

† Combined latencies: pattern is constant for NaCl or sorbitol.

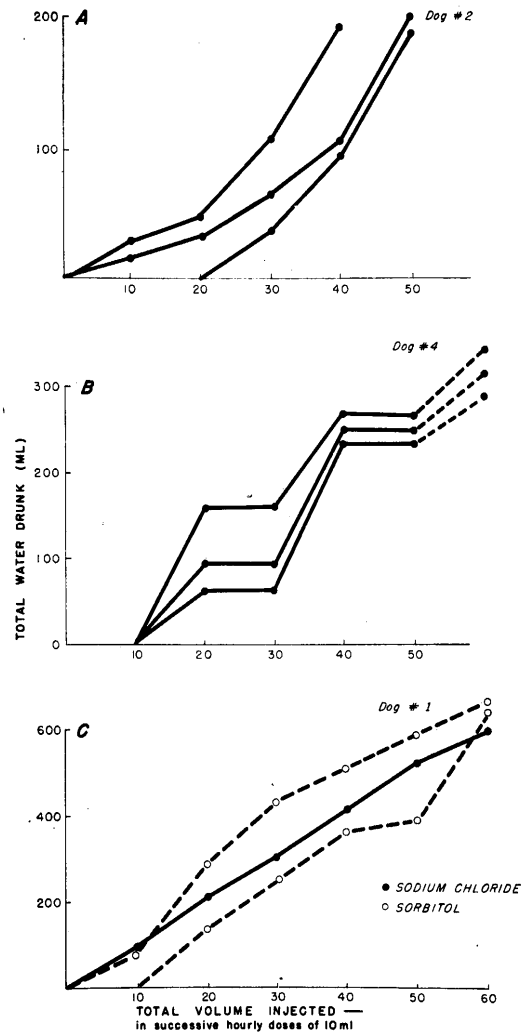

FIG. 1. Individual responses. Each curve represents responses to hourly injections on a single day.

A. Tendency for low responses to early stimuli; then successively larger intakes. (Dog #2)

B. Alternate hourly stimuli are refractory or much delayed but affect the responses to the succeeding ones. (Broken lines indicate drinking done 1-2 hr after last stimulus.) (Dog #4)

C. Consistent successive intakes, with compensation for occasional refractory periods. (Dog #1)

No. 5 had a much shorter latency than Dog No. 3, but a lesser response (Table I). Furthermore, long latency responses to NaCl were not associated with a decrease in volume. From the available data it is not apparent whether the responses to sorbitol would, as might be expected, be smaller with longer latencies. In dogs No. 2 and 4 even the rapid sorbitol responses appear to be less than those to NaCl.

TABLE III. Latency Alteration Following Recovery from Nembutal Anesthesia.*

Dog	Total responses	100% time range	Normal responses in this range	Normal medium latency
		(min)	%	(min)
1†	3	1 –3	31	4–5
2†	5	2 –3.5	26	5–7
4‡	4	.3–1	13	20

* Experiments performed 2 days after administration of Nembutal (6%, 0.5 cc/kg).
† Sorbitol injected.
‡ Sodium chloride injected.

In experiments done on an animal the second day after it had been anesthetized with sodium nembutal (6%, 0.5 ml/kg body weight), the latencies were significantly reduced while the volumes drunk increased (Table III). The latency values became much more consistent with some reflection in the magnitude of responses as well.

Discussion. The relationship between relative effectiveness of NaCl and sorbitol in inducing drinking and the time taken by an animal to respond to the stimulus has not been established. A simple explanation is that the 2 substances are equally effective when response is prompt, but that when execution of the response is delayed, sorbitol is gradually inactivated, leaving a weaker excitatory state. The mechanism which could account for such a phenomenon as delayed responses is probably not at the level of the primary receptors. Concerning inhibitory signals to whichever brain centers integrate the afferent information, if passage of water out of the gut means decreasing an inhibitory signal to a "drinking center," while the ensuing fluid changes tend to decrease the excitatory one, a manifestation of imperfect synchrony might be seen as drinking several times instead of only once following an initial adequate stimulus, such as was often seen in these experiments. This circumstance would presumably occur when the inhibitory signal is weak and experimentally occurred when the initial draught had been a small one.

These experiments confirm previous observations(7) that drinking following hyper-

tonic stimulus falls quite short of isotonic dilution (here, 11.1 ml/ml NaCl or sorbitol). However the fact that dogs quickly replace water lost through panting to within 1% of their body weight(9), indicates that drinking induced by a given degree of overconcentration of body fluids may differ according to how it is produced. Agreement might be improved if the dose size were increased and/or the doses followed one another more quickly. The considerable variation from animal to animal is probably a reflection of the fact that the "settings" of how accurately or quickly water deficit is made up may be a highly individual variable.

The drinking response has been shown to be related to the "effective strength" of the stimulus. With a dose twice as concentrated (7,8) much closer agreement in the average responses of individuals was noted, and latency values for each animal showed much less fluctuation than seen here. At 5% NaCl (8) not all dogs respond to doses of 10 ml. If the volume is greatly increased, "immediate"-drinking dogs may still respond at 1.5% salt, but the latencies increase greatly with dilution of the dose. Apparently the average latency of an individual to a given dose is some measure of the "strength" of that dose to that animal. It will lengthen as the stimulus strength decreases toward threshold and at the same time, being less stable against uncontrolled variations affecting the thirst pathways, will become less consistent. However, long-latency animals do not generally respond more weakly in amount per given dose than do the immediate drinkers, nor is there a tendency for the animals to drink less after a salt stimulus when the latency has happened to be longer. But decreased latency did appear together with greater response and improved consistency with exposure to nembutal.

The apparent relationship of sorbitol effectiveness and latency could be checked by studying the variations of the NaCl: sorbitol response ratios when the over-all latency of an individual is altered and also with changes in single latency values. The mechanism of the initiation of the afferent impulses and the nature and extent of the nervous connections affecting the reflex must now be examined more directly. However, this type of "complete behavior" study has shown some of the over-all features of the reaction.

Summary. Osmotically equivalent hypertonic solutions of sodium chloride and sorbitol were injected hourly into the jugular veins of adult dogs; the drinking responses to each solution were compared for volume and latency. It is shown that over a period of hours an individual's volume intake produced by injection of a given volume of hypertonic solution tends to be constant while there is great variation in response to successive hourly stimuli. Individuals have characteristic latencies, though with a large range of variation at low stimulus strength. The alcohol can be as effective a stimulus as the salt, but it appears to be gradually inactivated, becoming proportionally less effective upon longer latency animals. While latency does not vary with magnitude of response, neither between a given dog's responses nor between individual averages, other tests made following recovery from nembutal produced increased drinking responses with shorter latencies.

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