TABLE I. Brown Fat Thermogenesis during Cold Exposure (4°C).[a]

	Rate of heat production (kcal/hour)
Intact rat[b]	4.24 ± .40
Interscapular pad	
dq_{cv}/dt	.043 ± .007
dq_{cn}/dt	.058 ± .004
dq/dt	.102 ± .007
Total brown fat	
dq/dt	.347 ± .013
	Contribution of brown fat (% total heat)
Interscapular pad	2.4 ± .1
Total brown fat	8.2 ± .2

[a] Means ± SE where n = 6. Definition of symbols: dq_{cv}/dt = rate of convective heat transfer; dq_{cn}/dt = rate of conductive heat transfer; $dq/dt = dq_{cv}/dt + dq_{cn}/dt$. Since q_f is essentially a time-independent step function, $dq_f/dt = 0$.

[b] From a caloric equivalent of 4.74 cal/ml of O_2.

only to the nonshivering thermogenesis of the animal.

Moreover, the heat production by the brown adipose tissue should be evaluated not only in terms of the amount of heat evolved, but also with respect to its distribution to the body heat sink. As has been previously emphasized (12), the topology and vascular relationships of the brown fat masses facilitate local application of their heat specifically to the vital organs of the thorax (5), the upper spinal cord (5,11), and the autonomic sympathetic chain (5).

Summary. Six adult rats, exposed to 5°C for 3–4 weeks and returned to a 26°C room for 10–14 days, were acutely prepared by insertion of thermocouples to measure temperatures of the colon, the interscapular brown fat, the arterial and venous blood perfusing the interscapular pad, and the inner and outer surfaces of the skin overlying the pad. From the temperature changes observed when the rats were reexposed to cold, the heat produced by the interscapular pad was calculated and extrapolated to that of the total brown fat. These values compared to the concurrently measured total caloric output indicated that during cold stress the intrinsic metabolism of brown adipose tissue accounted for 8.2% of the total heat production.

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The Lecithinase (α Toxin) Activity of Strains of *Clostridium perfringens** (32783)

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Nygren (1) has postulated that food poisoning due to *Clostridium perfringens* depends on the production of phospholipase C (lecithinase C, α toxin) by this organism, whereas Smith (2) has suggested that the ingestion of large numbers of the organism is responsible. Dack *et al.* (3) after feeding human

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volunteers over 10^9 viable cells of *C. perfringens* concluded that these organisms did not cause food poisoning. It is, therefore, still not clear if food poisoning caused by *C. perfringens* is due to infection or intoxication. The role of lecithinase in the mechanism of pathogenicity has also yet to be elucidated.

Hobbs *et al.* (4) stated that organisms responsible for food poisoning are atypical strains of *C. perfringens* type A differing from classical infectious strains by low levels of lecithinase (α toxin), no θ toxin (hemolysin), and either non- or α hemolytic on horse blood agar. Furthermore, these strains produced heat resistant spores (able to withstand 100°C for at least 1 hour). However, Hall *et al.* (5) reported that *C. perfringens* outbreaks in the United States were not restricted to strains meeting the criteria described by Hobbs *et al.* (4). They stated that any strain of *C. perfringens* from incriminated food must be considered in the etiology of the food poisoning outbreak (5). Weiss and Strong (6) found an inverse relationship between heat resistance of the spores of *C. perfringens* and toxigenicity of their vegetative cells.

It is evident that more research is needed to determine if there are "strains" of *C. perfringens* responsible for food poisoning outbreaks. If such strains exist, we need to know the properties associated with these strains. Since heat resistance of spores and low lecithinase activity has been implicated in strains isolated from food and food poisoning outbreaks (4,6,7), we studied the relationship between these properties in various strains of *C. perfringens*. Furthermore, we examined the variation in lecithinase production to determine if this was a stable or variable property.

Materials and Methods. Organisms. Eight strains of type A *C. perfringens* were used. Strains BP6K and PB6H, classical infectious strains (sources of isolation unknown), and strain F106, isolated from human feces, were obtained from the National Institutes of Health, Bethesda, Maryland. Strain A48, from chicken broth, strain A91, from a pathological specimen, and Hobbs' serotype 3 (originally National Collection of Type Cultures 8239), from boiled salt beef, were obtained from H. E. Hall, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio. Strain UM707, from dust, was obtained from St. Patrick Hospital, Missoula, Montana. Strain NCTC 8246, a classical infectious strain (source of isolation unknown) was obtained from R. Fuller of the National Institute for Research in Dairying, Shinfield, Reading, Berkshire, England.

Culture media. The medium for α toxin production contained per liter 20 gm of Trypticase (Baltimore Biological Laboratory), 5 gm of yeast extract (Difco), 2.5 gm of soluble starch (Baker and Adamson), 1 gm of saccharose (Difco), 0.16 gm of K_2HPO_4, 0.1 gm of $MgSO_4 \cdot 7H_2O$, and 0.1 gm of $Fe_2(SO_4)_3 \cdot nH_2O$ (pH 6.8). Viable cell counts were made on brain heart infusion agar (Difco). Rapidly growing cultures of *C. perfringens* for lecithinase activity were obtained by incubating the organisms in fluid thioglycollate medium (Difco).

Culture method. For α toxin production, 1 ml of an 18-hour thioglycollate culture of *C. perfringens* was inoculated into medium and incubated for 5 hours at 46°C.

Preparation of culture filtrates. Cultures were centrifuged for 45 min at 4000 rpm. The supernatant liquid was filtered and stored at 4°C. Lecithinase activity was determined within 2 hours after the cells were removed.

Counting method. Decimal dilutions were carried out to a final dilution of 10^{-12} using the medium for toxin production as diluent. Anaerobiosis was produced by activating a Gaspak (BBL, no. 06-110) in the incubation jar. The colonies were counted after 24-hours incubation at 37°C and the number of viable cells/ml was calculated.

Assay of α toxin. A modification of the method of Van Heyningen (8) was used for rapid determination of the lecithinase activity in culture filtrates. Egg yolk saline, the substrate, was freshly prepared every 5 days. A standard solution of enzyme was prepared for each determination by dissolving 0.1 mg/ml of lecithinase C (Nutritional Biochemicals Corporation, Cleveland, Ohio) in cold (4°C) 0.05 *M* Tris buffer pH 7.2. Varying amounts (10–200 μg) of lecithinase were added to assay tubes containing 2.0 ml of

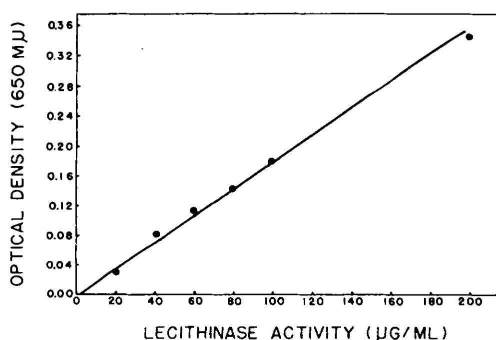

FIG. 1. Standard assay curve for determining lecithinase activity.

egg yolk saline and 0.4 ml of 0.1 *M* $CaCl_2 \cdot 2H_2O$. The total volume was adjusted to 5.4 ml with 0.05 *M* Tris buffer pH 7.2. The data obtained were used to construct the standard assay curve for lecithinase activity.

The enzyme activity of the strains of *C. perfringens* was determined by placing 1 ml of each culture filtrate into the assay tubes. The control tube for each determination contained 0.2 ml of standard lecithinase solution and 0.2 ml of undiluted *C. perfringens* type A diagnostic serum (Wellcome Research Laboratories, Beckenham, England) to inhibit enzyme activity. The assay tubes were incubated at 46°C for 15 min. Then 6.0 ml of cold (4°C) distilled water were added to each tube and thoroughly mixed. The contents were immediately transferred to glass cuvettes and read against the control at 650 mμ, with a spectrophotometer (Coleman Junior, model 6C). The lecithinase activity of each culture filtrate was interpolated from the standard assay curve (Fig. 1).

Results. The method employed for the assay of lecithinase was rapid and reproducible. The standard assay curve (Fig. 1) enabled us to quantitate the lecithinase activity present in the culture filtrates of different strains.

Table I lists the lecithinase activities of the 8 strains of *C. perfringens* studied and indicates considerable variation from strain to strain in the amount of lecithinase produced. The classical infectious strains, BP6K, NCTC 8246, and PB6H, produced more lecithinase than the other strains, but, the lecithinase activity of these three strains showed considerable variation from experiment to experiment. The standard deviation values indicated that there was more random error and a greater scatter of individual measurements around the mean by strains possessing high lecithinase activity. Strains that produced small quantities of lecithinase, namely, strains Hobbs 3, A91, and F106, were more consistent in quantity of toxin produced as evidenced by range of toxin production and standard deviation values (Table I).

Discussion. The toxigenicity of strains of *C. perfringens* was compared with data obtained by Nakamura and Converse[1] on the heat resistance of spores of these strains. In general, strains that possessed high toxigenicity also produced spores of relatively low heat resistance, (readily destroyed at 90°C in less than 30 min). Strains with low toxigenicity produced spores of high heat resistance (requiring more than 150 min at 90°C for destruction). The one exception, strain F106, a low toxigenic strain, produced spores with low heat resistance. This inverse relationship between lecithinase production and heat resistance of spores is in general agreement with the work of others (6,9). We noted, in addition, an interesting variation in toxigenicity between experiments in strains

TABLE I. Lecithinase (α Toxin) Activities of Eight Strains of *Clostridium perfringens.*

Strain	Mean viable cells/ml[a]	Lecithinase activity (μg/ml)		
		Range	Mean[a]	SD
BP6K	1.3×10^{11}	38–145	98.6	40.6
NCTC 8246	8.5×10^{11}	56–139	78.8	31.3
PB6H	9.5×10^{11}	37–107	65.6	23.3
A48	9.9×10^{11}	19– 30	25.8	4.1
UM707	8.3×10^{11}	10– 28	20.2	7.9
Hobbs 3	1.3×10^{13}	16– 20	18.8	1.6
A91	9.1×10^{13}	12– 15	13.8	1.1
F106	3.9×10^{13}	12– 15	13.5	1.1

[a] Calculated on the basis of 4 or more determinations.

[1] Nakamura, M., Converse, J. D., to be published.

of high toxigenicity, but not in strains of low toxigenicity.

Although the heat resistance of spores of *C. perfringens* is a genetically stable property (10), Weiss and Strong (11) reported that spores of selected strains of *C. perfringens* varied considerably in their heat resistance when different sporulation media were used to produce the spores. Toxigenicity, at least in strains of high toxigenicity, is also a variable property. This variation may be due to differences in the nutritional requirements for lecithinase production (12,13). Therefore, in examination of strains of high toxigencity, several determinations should be made to determine the level of lecithinase activity. A single experiment may yield the false conclusion that the strain is an intermediate or low toxigenic form.

Although heat resistance of the spores and level of lecithinase production are two properties used to distinguish food poisoning strains from classical type A strains, the present study indicates that these two criteria cannot be relied upon completely in the identification of food poisoning strains.

Summary. Lecithinase activity was determined for 8 strains of type A *C. perfringens* isolated from various sources. The 3 classical infectious strains of *C. perfringens* with spores of low heat resistance produced high lecithinase activity. In general, there was an inverse relationship between lecithinase activity and heat resistance of the spores. However, strains that produced high lecithinase activity had wider ranges and greater standard deviation values than strains with low levels of lecithinase.

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