THE PYROGENICITY OF BACTERIAL CONTAMINANTS FOUND IN BIOLOGIC PRODUCTS

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The increasing use of intravenous therapy, particularly blood plasma or other blood products, has served to bring into greater prominence the role of bacterial contamination as the cause of occasional thermal reactions. Information was therefore needed concerning the bacteria which are the principal offenders, including their pyrogenic characteristics. The role of bacteria in causing immediate thermal reactions following intravenous therapy has been known for many years, yet proper attention has only recently been directed towards the prevention of these reactions. A test for the detection of pyrogens in solutions for parenteral administration was included in the twelfth revision of the U. S. *Pharmacopoeia* (1942). The details of the test were formulated from the results of a collaborative study (McClosky *et al.*, 1943).

In the past reactions resembling shock following intravenous injection of aqueous solutions were generally named for the particular medication involved and, for example, were referred to as "salt fever," "protein fever," "glucose fever," and "salvarsan fever." However, there were some workers who more properly designated the reactions as "water fever," as the diluent used in all these preparations was water. As early as 1865 Billroth reported reactions following injections of some distilled waters. Since then numerous workers have definitely indicted water as containing the causative agent, and also have identified the agent as being of bacterial origin.

Hort and Penfold (1911, 1912a, 1912b, 1912c) further advanced the theory of the bacterial origin of pyrogen. They made these fundamental observations: (a) that pyrogenic substances were filterable, thermostable, and quantitative in nature; (b) that pyrogen-free saline injected intravenously into rabbits caused a fall in temperature; and (c) that two types of fever were induced in rabbits by bacterial suspensions. One was an immediate reaction which they designated a "fugitive fever," whereas the other was delayed and more prolonged. They also described a third type but this was a fever caused by actual infection.

Seibert (1923–24, 1924–25) confirmed the earlier observations and showed conclusively that pyrogens are of bacterial origin. Working with bacteria isolated from distilled water, Bourn and Seibert (1924–25) observed marked variations qualitatively and quantitatively in the pyrogenicity of different bacteria. Seibert and Mendel (1923–24a) demonstrated that the so-called protein fever induced by casein was of bacterial origin and was caused by contamination of the milk used in the preparation of the casein. They (1923–24b) also contributed to the evolution of the rabbit thermal test for the detection of pyrogens.

The term *pyrogen* has been applied to a bacterial substance which induces an immediate "fugitive fever," especially characterized by the reaction displayed by *Pseudomonas*. This concept has been emphasized by the reports of Banks (1934) and of McClosky *et al.* (1943). The former used *P. ureae* and *P. scicca* and the latter *P. aeruginosa*. Banks also used *Staphylococcus albus* and *Bacillus subtilis* and reported that these bacteria were nonpyrogenic since they induced only a delayed fever similar to that reported by Hort and Penfold. Co Tui and Schrift (1942a) demonstrated that pyrogen production is not confined to any one group of organisms, and is more widespread than was first envisaged. They had previously (1939) suggested that the "chill and fever producing substances in sera may be pyrogens." A clinical reaction to 75 ml of liquid human plasma contaminated with *S. albus* was reported recently by Weinstein (1942).

It is evident from this review of the literature that, whereas considerable work has been done with pyrogens produced by microorganisms present in diluent water, there has been little study of the pyrexial characteristics of other organisms present in biologic products as contaminants derived from sources other than diluent water.

This report primarily is concerned with the pyrogenic properties of those microorganisms isolated from blood or plasma during the various stages of the processing to dried normal human plasma. The problem was to determine the quantitative and qualitative nature of the thermal response of rabbits (a) to the living bacteria, (b) to heat-killed bacteria, and (c) to whole cultures grown in serum. This information will be useful as a guide in determining standards for biologics intended for human therapy.

MATERIALS AND METHODS

Materials. Forty-nine cultures were received from 8 processing laboratories widely distributed throughout the United States. From these, 28 representative cultures were selected for study. These included staphylococci, micrococci, streptococci, gram-negative bacilli, and sporeforming and nonsporeforming gram-positive bacilli. Some of the cultures used have not been completely identified.

Sodium chloride solution, 0.85 per cent, which was used as the diluent, was prepared with one lot of sodium chloride (A.C.S.) proved by animal test to be pyrogen-free, and with water freshly redistilled in a glass still. Several lots were prepared at one time and sterilized in the autoclave. Each lot was tested and accepted for use only if the rabbit thermal response induced was characteristic of pyrogen-free saline.

Methods. The thermogenic properties of the test material were determined by the pyrogen test as described in the U. S. *Pharmacopoeia* XII, except as detailed below:

Rabbits weighing from 2,000 to 3,500 grams were used. Smaller rabbits were not used because in several preliminary tests they were less responsive to small amounts of pyrogen. The animals selected for the test were ones having a normal preinjection temperature between 39.0 C and 39.8 C; they

were arranged so that the average temperatures of the groups were comparable. The test dose of 15 ml per kilogram of the rabbit's body weight closely approximates the maximum amount of plasma, on a weight basis, that would be likely to be administered to a patient within a short time. Six hourly posttreatment temperatures were taken beginning one hour subsequent to the injection. In each day's tests we studied the animal thermal response to several bacterial suspensions, usually four, and to one normal saline. The single saline group served as the control for the diluent used in preparing the bacterial suspension,

TABLE 1

Temperature deviation induced in rabbits by saline and by Aerobacter cloacae PC3B

TEST MATERIAL	RABBIT NUMBER	NORMAL TEMPERATURE	TEMPERATURE DEVIATION								
			Hourly						High above normal	High above control	Peak above control
			1	2	3	4	5	6			
		C	C	C	C	C	C	C	C	C	C
Saline control..	742	39.7	−0.5	−0.3	−0.3	−0.2	−0.1	−0.1	−0.1		
	666	39.6	−0.1	−0.2	−0.1	−0.1	−0.1	−0.1	−0.1		
	667	39.6	−0.3	−0.6	−0.3	−0.3	−0.3	−0.3	−0.3		
	675	39.8	−0.3	−0.3	−0.3	−0.2	−0.2	−0.2	−0.2		
	669	39.7	−0.2	−0.4	−0.2	−0.2	−0.2	−0.2	−0.2		
Average			−0.28	−0.36	−0.24	−0.20	−0.18	−0.18	−0.18		
Bacterial dilution 5×10^{-5} 70,000 per ml	678	39.6	0.1	0.5	0.4	0.9	1.0	0.8	1.0		
	679	39.7	0.8	1.0	0.8	1.2	1.0	0.6	1.2		
	671	39.8	0.8	1.3	1.1	0.5	0.2	0.1	1.3		
	673	39.6	0.5	1.2	1.7	2.1	1.7	1.2	2.1		
	674	39.3	0.8	1.0	0.5	0.5	0.4	0.4	1.0		
Average			0.60	1.0	0.9	1.04	0.86	0.62	1.32	1.50	1.24
5×10^{-6} 7,000 per ml	668	39.7	−0.4	0.1	0.0	0.0	−0.1	−0.2	−0.1		
	676	39.4	0.2	0.1	0.2	0.1	0.0	0.0	0.2		
	670	39.8	0.0	−0.1	−0.1	−0.1	0.0	0.0	0.0		
	672	39.0	0.3	0.7	0.5	0.6	0.6	0.6	0.7		
	688	39.5	0.1	0.0	0.0	0.0	0.0	0.0	0.1		
Average			0.04	0.16	0.12	0.12	0.1	0.08	0.22	0.40	0.52

and as the pyrogen-free control for the day's tests. The saline group also served as the control for the technique and other external factors, including temperature and humidity, which might unfavorably influence the test animals. This was quite important as the animal room was not adequately air-conditioned.

The hourly and the maximum deviations from the normal pretreatment temperature were calculated for each rabbit and averaged for all the animals receiving the same test material. The pyrexial response induced by the bacterial suspensions then was computed by three procedures. The first procedure, *high above normal*, which is the U.S.P. method, bases the computation on the

temperature deviation from the pretreatment normal temperature. The second method, *high above control*, records the difference between the maximum temperature deviation induced by the test material and that of the pyrogen-free control. The third procedure, *peak above control*, computes the pyrexia as the difference between the temperature induced by the test solution at the time (hour) of maximum temperature rise and the temperature of the pyrogen-free controls at the corresponding hour.

In table 1, a typical protocol, the three methods of expressing the pyrexia induced by two dilutions of a living bacterial suspension, *Aerobacter cloacae*, with saline control are illustrated. The thermal response with the higher concentration derived by the three methods were 1.32 C, 1.50 C, and 1.2 C, and with the lower concentration they were 0.22 C, 0.40 C, and 0.52 C, respectively.

TABLE 2

Pyrexia induced by living bacteria—gram-negative bacilli

CULTURE				TEMPERATURE DEVIATION		
Species		Dilution	Bacteria per ml	High above normal	High above control	Peak above control
				C	C	C
Aerobacter cloacae	PC7	5×10^{-6}	2,500	0.04	0.12	0.15
		1×10^{-4}	50,000	1.36	1.44	1.45
Escherichia freundii	PC23	1×10^{-5}	5,000	0.32	0.36	0.34
Pseudomonas ovalis	PC25	1×10^{-5}	300	−0.03	0.05	0.08
		1×10^{-4}	3,000	0.58	0.66	0.68
Species (?)	PC40	1×10^{-6}	1,600	0.44	0.36	0.54
		1×10^{-5}	16,000	1.22	1.14	1.35

PYROGENICITY OF LIVNG BACTERIA

In the first stage of the study the qualitative and quantitative pyrogenic characteristics of living bacteria, uninfluenced by culture media, metabolic products, heterologous protein, etc., were studied. Thus the potential pyrogenic contamination due to a specific living organism or to the number of living bacteria could be evaluated.

Cultures were grown for 18 hours on agar slants from which the bacteria were removed with a platinum loop, suspended in saline, and standardized to a density corresponding to 500 ppm of silica. Serial saline dilutions, usually tenfold, were prepared from the basic bacterial suspension. The number of viable bacteria per ml was determined by cultural methods. Serial dilutions of the suspension of living bacteria were injected intravenously into rabbits to determine the minimum number of bacteria, or the bacterial suspension, that would induce (a) slight—i.e., 0.3 to 0.5 C—and (b) marked rise in temperature. The results obtained are grouped and presented according to the morphology of the bacteria.

Gram-negative bacilli. The gram-negative bacilli, reported in table 2, were the most pyrogenic of all the bacteria studied. Approximately 2,000 to 5,000 bacteria per ml, irrespective of the strain, were capable of inducing a slight rise in temperature, and 10 to 20 times that number induced a marked rise.

The bacteria of the coli-aerogenes group (figure 1), induced an immediate rise in temperature, which reached its maximum at the second hour, returning to normal within the test period. This is a typical so-called *pyrogen reaction.* The unidentified culture, PC40, reported in table 2 but not illustrated, induced a

TABLE 3

Pyrexia induced by living bacteria—gram-positive bacilli

CULTURE				TEMPERATURE DEVIATION		
Species		Dilution	No. of bacteria	High above normal	High above control	Peak above control
			per ml	*C*	*C*	*C*
B. subtilis group						
B. cereus	PC3A	10^{-3}	10,000	0.18	0.20	0.26
		10^{-2}	100,000	0.84	0.86	0.96
B. tumescens	PC36	10^{-3}	100,000	0.24	0.19	0.35
		10^{-2}	1,000,000	1.46	1.41	1.47
Diphtheroids						
	PC18	10^{-4}		0.30	0.22	0.24
		10^{-3}		1.80	1.60	1.69
		10^{-2}		1.76	1.70	1.66
	PC20	10^{-3}		0.22	0.22	0.20
		10^{-2}		1.72	1.72	1.70
	PC32	10^{-3}		0.25	0.20	0.22
		10^{-2}		1.54	1.50	1.42
	PC33	10^{-3}		0.12	0.08	0.30
		10^{-2}		0.40	0.36	0.42

rather slow rise in temperature, which reached its peak between the fourth and fifth hours. In contrast, however, with cultures of *Pseudomonas ovalis* there was an initial depression temperature followed by a rather slow rise, reaching the maximum later in the observation period (figure 2).

Gram-positive bacilli. The gram-positive bacilli (table 3) were less pyrogenic than the gram-negative by approximately a hundredfold. The two organisms of the *Bacillus subtilis* group reacted similarly, inducing the same type of temperature curve (figure 3), but *Bacillus tumescens* was apparently more active than *Bacillus cereus.* There was an immediate drop in the animal temperature followed by a sharp rise which reached the maximum about the third hour. The four diphtheroid cultures showed considerable variation in pyrogenicity, more

than that noted in the *B. subtilis* group. On the basis of serial bacterial suspensions prepared from the 18-hour agar slant cultures, which were more ac-

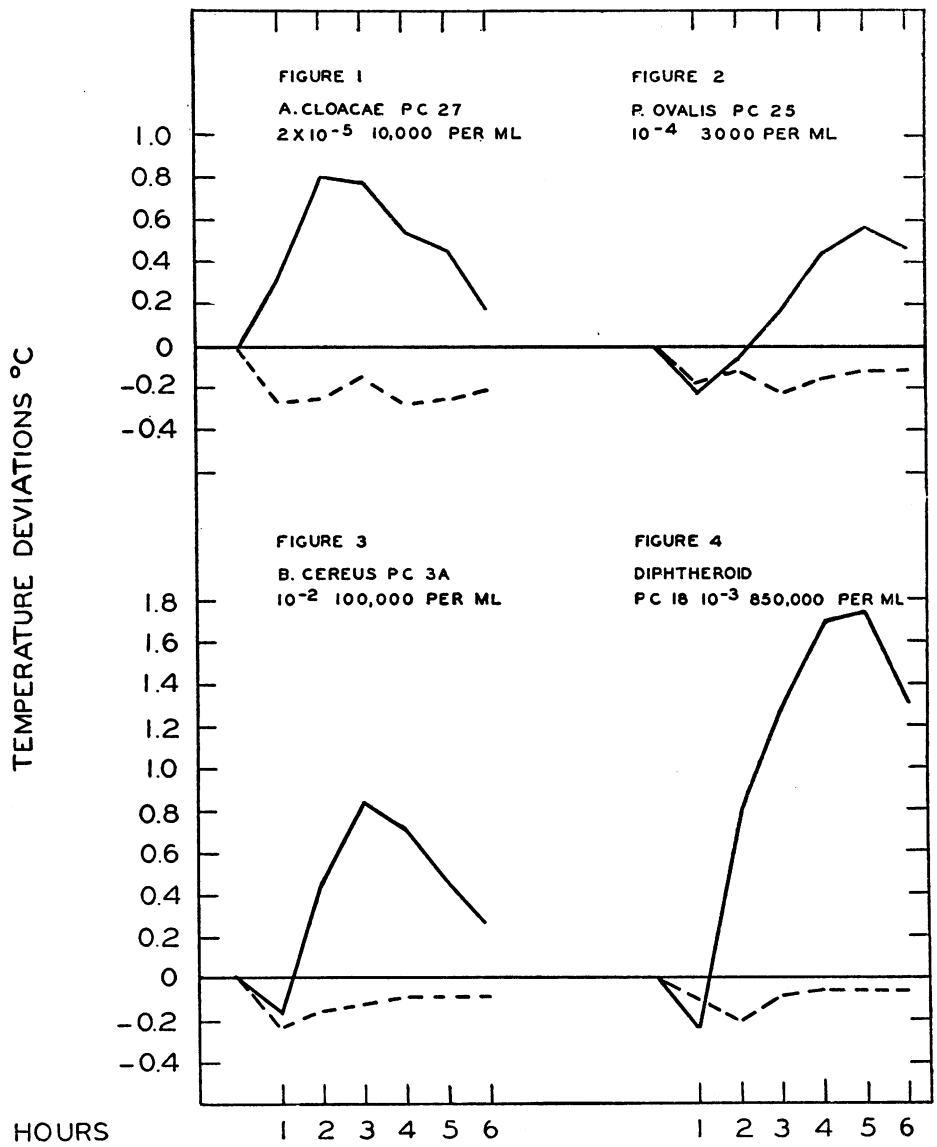
TABLE 4

Pyrexia induced by living bacteria—gram-positive cocci

CULTURE				TEMPERATURE DEVIATION		
Species		Dilution	No. of bacteria	High above normal	High above control	Peak above control
			per ml	*C*	*C*	*C*
Staphylococcus						
S. albus	PC39	2×10^{-4}	60,000	0.18	0.18	0.25
		10^{-3}	300,000	1.32	1.32	1.33
	PC30B	10^{-3}	130,000	0.56	0.51	0.52
		10^{-2}	1,300,000	1.32	1.27	1.22
S. epidermidis	PC24	10^{-3}	400,000	0.08	0.06	0.14
		10^{-2}	4,000,000	1.20	1.18	1.26
S. aerogenes	PC1	10^{-3}		0.12	0.22	0.24
		10^{-2}		0.24	0.34	0.40
Streptococcus						
S. liquefaciens	PC16	5×10^{-4}	200,000	0.30	0.24	0.28
		5×10^{-3}	2,000,000	1.64	1.58	1.66
S. faecalis	PC29	10^{-3}	200,000	0.18	0.08	0.10
		10^{-2}	2,000,000	1.12	1.02	0.90
S. equinus (?)	PC41	2.5×10^{-3}	520,000	0.00	0.23	0.40
		10^{-2}	2,600,000	1.70	1.93	2.02
(?)	PC22	10^{-3}	200,000	0.26	0.16	0.10
		10^{-2}	2,000,000	0.48	0.38	0.30
Micrococcus						
M. epidermidis	PC11	10^{-3}	200,000	0.42	0.46	0.66
		10^{-2}	2,000,000	1.84	1.88	2.00
M. flavus	PC17	10^{-3}	90,000	0.20	0.30	0.20
		10^{-2}	900,000	0.58	0.68	0.64
M. subflavescens	PC30C	10^{-3}	80,000	0.28	0.38	0.26
		10^{-2}	800,000	1.58	1.68	1.60
M. candidus	PC14	10^{-3}	70,000	0.33	0.43	0.48
		10^{-2}	700,000	1.00	1.10	1.14

curate than counts of the living bacteria because of clumping, culture PC33 in 10^{-2} dilution was as thermogenic as PC18 in 10^{-4} dilution. The delayed type of pyrexia induced by the diphtheroids (figure 4) was similar to that induced

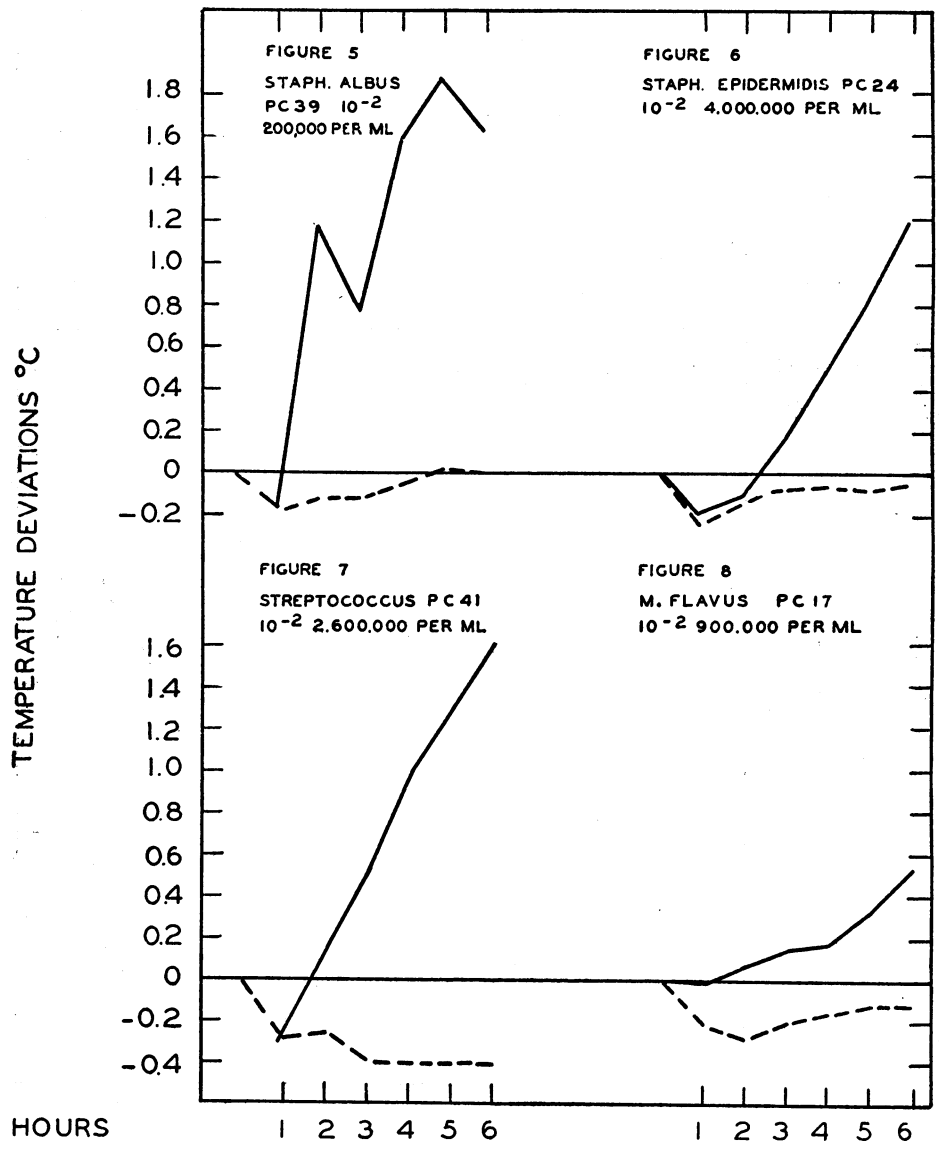
by those of the *B. subtilis* group except that the maximum thermal response was further delayed, reaching the peak the fourth or fifth hour.



FIGS. 1–4

Gram-positive cocci. The gram-positive cocci, *Staphyloccoccus*, *Streptococcus*, and *Micrococcus*, reported in table 4 were the least thermogenic of the bacteria studied. The thermogenicity of these bacteria varied, not only within the group as a whole but within each genus. All cultures induced the delayed type of animal response.

Of the *Staphylococcus* cultures studied, *S. albus* was approximately ten times more thermogenic than *S. epidermidis*, and *S. aerogenes* was the least reactive



FIGS. 5–8

of all the bacteria. With *S. albus* (figure 5) there was an immediate 1-hour depression in the rabbit temperature followed by a sharp and accelerated rise, whereas with *S. epidermidis* (figure 6) the initial depression lasted over 2 hours followed by a very slow rise in temperature which apparently did not reach its

peak during the observation of 6 hours. *S. aerogenes* induced a response similar to that of *S. epidermidis*.

Five strains of *Streptococcus*, 3 enterococci and 2 unidentified, were studied, and of these 4 are reported. The *Streptococcus liquefaciens* PC16 was apparently the most pyrogenic. The thermal response induced by the *Streptococcus* PC41 (figure 7) was an initial 1-hour depression temperature followed by a

TABLE 5

Influence of heating on the thermogenicity of saline suspensions of bacteria

CULTURE		NUMBER OF BACTERIA	TEMPERATURE DEVIATION*		
			Living	Heated	
				60 C	120 C
		per ml	*C*	*C*	*C*
A. cloacae	PC3B	90,000	1.64	1.48	1.34
	PC27	6,000	1.12	1.16	
E. freundii	PC23	5,000	0.34	0.40	
P. ovalis	PC25	3,000	0.68	0.36	0.15
Species?	PC40	19,000	0.84	0.84	0.36
Diphtheroid	PC20	1,800,000	1.70	0.50†	0.84
B. cereus	PC3A	100,000	0.96	0.40‡	0.22
S. liquefaciens	PC16	2,000,000	1.66	1.18	1.08
Streptococcus (?)	PC41	2,600,000	2.02	0.61	
		3,000,000		0.78	0.76
S. albus	PC30B	1,300,000	1.22§	1.65	0.88
S. epidermidis	PC21	3,000,000		0.62	0.14

* Peak above control.
† Three rabbits.
‡ Not sterile.
§ Maximum rise had not been reached at the sixth hour.

gradual rise which continued during the entire observation period but was normal the following morning.

Of the 4 strains of *Micrococcus* reported, 3 were approximately equal in thermogenicity, but the fourth, *M. flavus* PC17, was less reactive. The temperature response induced by cultures of *M. flavus* (figure 8) is characteristic of the delayed type of pyrexial response induced by micrococci.

It is interesting to note that had the observation period been terminated at the end of 3 hours, the animal thermal test of living cultures of *P. ovalis*, *S. epidermidis*, *Streptococcus* PC41, and *M. flavus* (figures 2, 6, 7, and 8) would have been considered nonpyrogenic on the basis of the 0.6 C temperature rise.

PYROGENICITY OF HEAT-KILLED BACTERIA

The second stage of the work was concerned with the influence of killing with heat upon the pyrogenicity of bacteria, both as to the intensity and the nature of the animal thermal reaction. This phase of the study was accomplished by comparing the pyrogenicity of living bacteria with comparable cultures killed by heating at 60 C for 30 minutes and 121 C for 15 minutes. The cultures

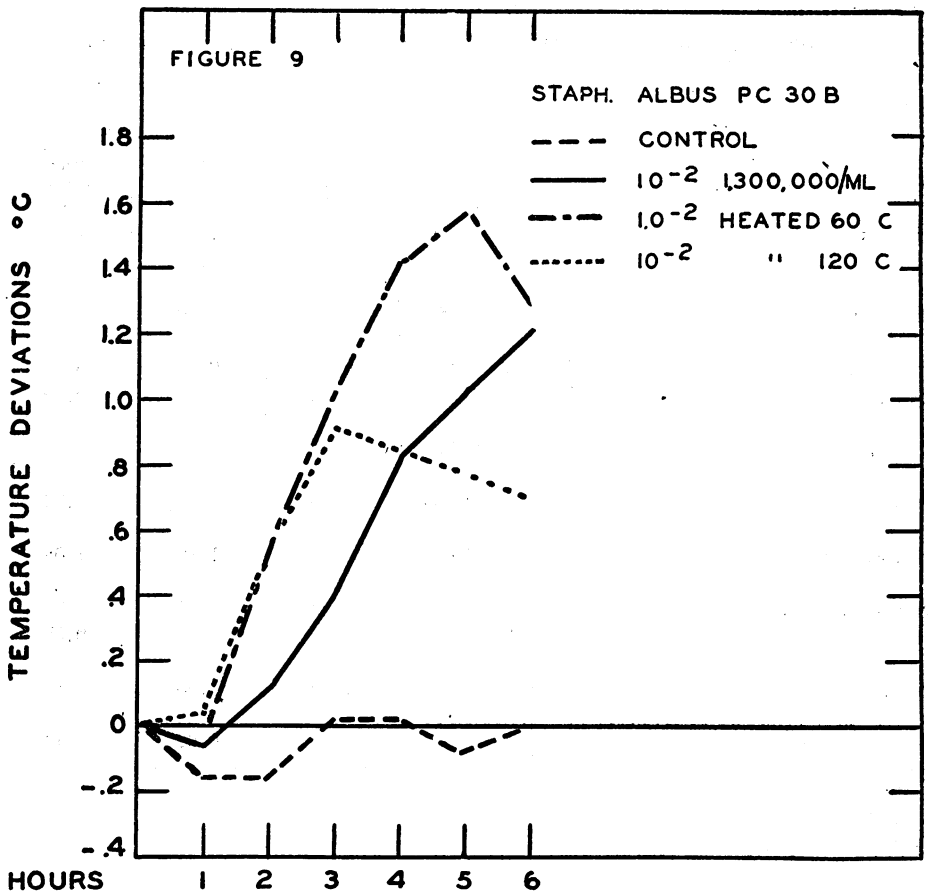


FIG. 9

were grown and the serial saline suspensions of the bacteria were prepared by the method previously described.

Typical results obtained with living cultures and heat-killed cultures at 60 C and 121 C, which represent the morphological groups which induced different types of febrile response, are reported in table 5. In general, the thermogenicity of the bacteria was reduced and altered by killing with heat.

The pyrogenicity of the gram-negative bacilli, with the exception of *P. ovalis*, was uninfluenced by heating at 60 C, and autoclaving brought about only a slight decrease. The culture of *P. ovalis*, the several cultures of the gram-pos-

itive bacilli, and the staphylococci were definitely reduced in pyrogenicity by heating at 60 C and still further reduced by autoclaving. The streptococci, however, were greatly reduced in thermogenicity by heating, but the loss was approximately the same at both temperatures.

The nature of the animal thermal response also was modified. The delayed type of temperature curve characteristic of the living organisms was observed with the heat-killed cultures, but the thermal reaction was accelerated, the latent or "lag" period being shortened and the pyrexia cycle completed more

TABLE 6

Comparison of thermogenicity of cultures grown in rabbit serum with cultures grown on agar

SPECIES		CULTURE GROWN IN RABBIT SERUM				CULTURE GROWN ON AGAR FOR 18 HOURS		
		Age	Dilution of serum culture	No. of viable bacteria	Temperature deviation*	Dilution of bacterial suspension	No. of bacteria	Temperature deviation
		days		*per ml*	*C*		*per ml*	*C*
A. cloacae	PC27	2	10^{-5}	? 1,000	0.64	10^{-5}	6,000	0.94
E. freundii	PC23	12	10^{-7}	45	0.19			
			10^{-6}	450	0.30			
			10^{-5}	4,500	1.11	10^{-5}	5,000	0.34
B. cereus	PC3A	12 and 3 mixed	6.4×10^{-3}	4,000	0.31	10^{-3}	100,000	0.26
			6.4×10^{-2}	40,000	0.85	10^{-2}	1,000,000	0.96
Diphtheroid	PC20	7	10^{-3}	16,000	0.14	10^{-3}	1,800,000	0.20
			10^{-2}	160,000	0.64	10^{-2}	18,000,000	1.70
S. epidermidis	PC21	14	10^{-3}	20,000	0.58	10^{-3}	400,000	0.08
			10^{-2}	200,000	1.62	10^{-2}	4,000,000	1.84

* Peak above control (1:100 dilution of uninoculated rabbit serum).

quickly. A typical illustration of the influence of heating at 60 C and 121 C on the thermogenicity of bacteria is shown in figure 9. It may be seen that the maximum response induced by the living bacteria, *S. albus* PC30B, was not reached during the observation period, whereas the maximal pyrexia induced by heat-killed cultures at 60 C and 121 C were at the fifth and third hour, respectively.

PYROGENICITY OF WHOLE SERUM CULTURES OF BACTERIA

The third part of the study concerns the pyrogenicity of bacteria grown in normal rabbit serum, the serum containing not only the living bacteria but also the products of metabolism. These experiments offered a means of appraising the possible pyrogenicity of blood or blood products contaminated with these bacteria and their products.

The bacteria were grown in normal rabbit serum for varying periods as designated in the table. The serial saline dilutions were prepared from the whole serum culture.

In table 6 is recorded the dilution of the serum cultures which induced quantitative thermogenic response compared with the dilution of the saline suspension of living bacteria grown on agar which induced comparable responses. The serum culture dilution contained only 1/10 to 1/25 as many viable bacteria as

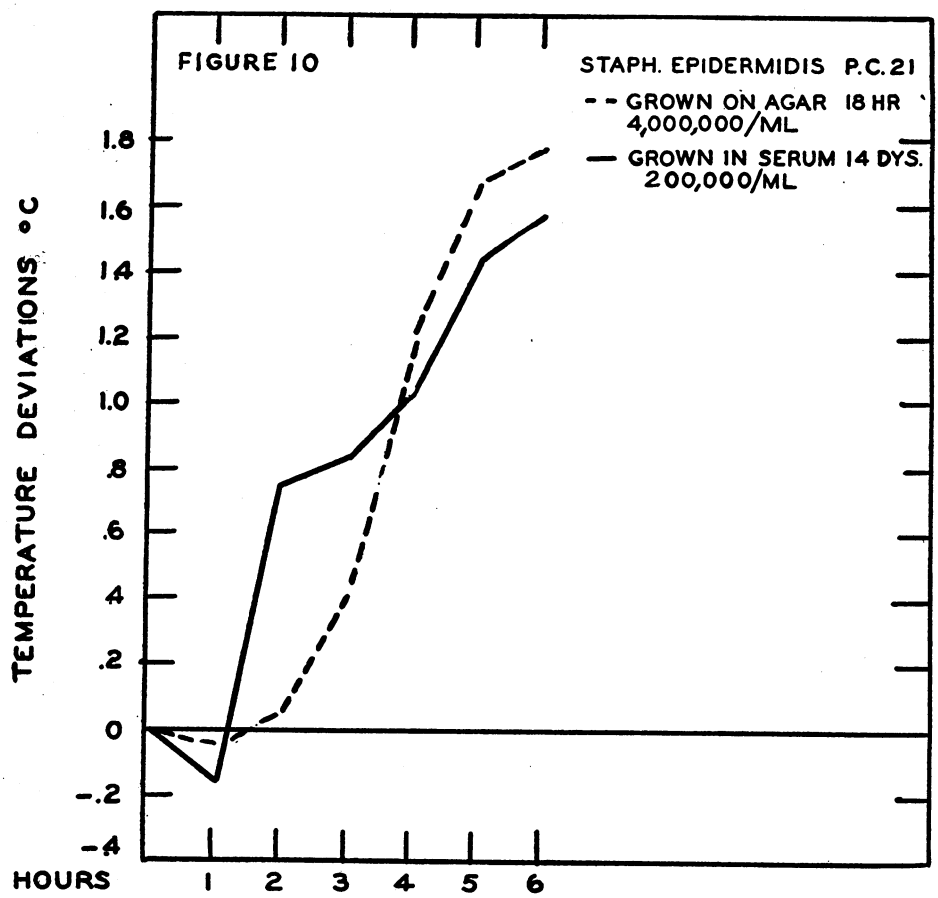


FIG. 10

the suspension of the bacteria grown on agar. The culture of *Escherichia freundii*, the most thermogenic of the serum cultures studied, offers the best illustration of the quantitative characteristic of serum cultures. Serial dilutions containing 45, 450, and 4,500 bacteria per ml induced rises in temperature of 0.19 C, 0.30 C, and 1.11 C, respectively. The thermal responses induced by the serum culture dilution containing 450 bacteria per ml and by the suspension of agar cultures containing 5,000 bacteria per ml were equal, 0.30 C and 0.34 C, respectively.

The nature of the animal thermal response to serum-grown cultures was similar to that induced by heat-killed cultures. The temperature rise was accelerated, and in those cultures which induced the delayed type of pyrexia, the depression or "lag" phase was shortened and the pyrexia cycle, generally, was completed more quickly. As illustrated in figure 10, the pyrexia induced by serum-grown cultures of *S. epidermidis* was accelerated as compared with the delayed pyrexia induced by the suspension of living culture grown on agar.

ANIMAL RESPONSE TO PYROGEN-FREE SALINE

The pyrogen-free saline control in practically every instance, as originally reported by Hort and Penfold, induced a depression from the preinjection normal temperature. Therefore the normal physiological reaction of the rabbit to injection of pyrogen-free saline (see table 1 and the broken lines in figures 1 to 9, inclusive) is depression of the normal temperature.

DISCUSSION

The thermogenic properties of the living bacterial cells of 28 cultures isolated from blood or plasma by the several processing laboratories have been studied. All were thermogenic but varied considerably in their quantitative and qualitative reactions. In general, the bacilli were more thermogenic than the cocci, with the gram-negative bacilli the most pyrogenic. It is important to note that of all the cultures studied only those of the coli-aerogenes group (figure 1) induced the immediate or "fugitive" fever which has been considered by other workers to be the typical pyrogen reaction. All other bacteria induced a delayed type of pyrexia. The maximum pyrexial response induced by some cultures may not have been reached, as the temperatures were still rising at the end of the 6-hour observation period (figures 6, 7, and 8). In each instance, however, the temperature was normal the following morning, and no infections developed.

Living bacteria were more pyrogenic than the corresponding heat-killed cultures in comparable dilutions, except the bacteria of the coli-aerogenes group, which were not significantly influenced. The loss in pyrogenicity was greater in the cultures killed by autoclaving (121 C) for 15 minutes than in those heated at 60 C for 30 minutes. A significant influence of heat is the acceleration of the delayed type of pyrexial reaction. This would indicate that heat-killing the bacteria caused an earlier release of the pyrogenic substances.

Bacteria grown in normal rabbit serum, on the basis of bacterial count, were at least tenfold more pyrogenic than saline suspensions of young agar cultures. The serum culture contained not only the viable organisms but also the metabolic products, including any soluble pyrogenic substances. Therefore, the number of organisms alone is not a reliable index of the safety of the product.

One of the significant observations is that the pyrogen-free saline control induced a depression in the normal rabbit temperature. This would suggest that the evaluation of the pyrogen test should be predicated upon the normal physiological response of the rabbit to injection with a pyrogen-free control and not upon the pretreatment normal temperature as is now recommended by

the U. S. *Pharmacopoeia*. Another observation is that the quantitative characteristic of pyrogens, as reported for the bacteria studied, indicates that a slight febrile response in rabbits, of less than the 0.6 C temperature elevation permitted by the *Pharmacopoeia* XII test, is in fact due to pyrogen contamination. This marginal amount of pyrogen may be sufficient to cause clinical reactions in humans, as Co Tui and Schrift (1942b) have observed that the rabbit is only one-third as sensitive to pyrogens as man.

In a recent report, Co Tui (1944) recommended that the official test be more quantitative and suggested the use of several test doses to insure freedom from marginal pyrogen contamination.

The 3-hour observation period of the U. S. *Pharmacopoeia* test may not be sufficient to obtain the maximum fever response to all pyrogens. It is not suggested that the extracted or purified pyrogens of these or any other microorganisms would induce the delayed reaction. However, until the characteristics of pyrogens are more fully elucidated, it is indicated that the observation period of the animal thermal test should be continued until the maximum response has been reached or until the temperature curve has definitely entered the downward phase.

CONCLUSIONS

Twenty-eight microorganisms isolated from contaminated blood or blood plasma during the processing of dried normal human plasma were all capable of inducing fever in rabbits but varied considerably in their thermogenic characteristics, both quantitatively and qualitatively.

The count of bacteria in a contaminated blood product does not furnish an index of pyrogenicity.

It is indicated that the animal thermal test should be predicated upon a pyrogen-free control, and the temperature elevation above that induced by the control should be considered as being caused by pyrogen contamination.

The observation period of the thermal test should be continued until definite evidence of pyrogenicity or nonpyrogenicity has been established.

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