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Production of Pyrogen in Gum Acacia by Bacteria.

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The occurrence of a "reaction" consisting of fever and chills (shivering) following the intravenous use of acacia is one of the major deterrents to its widespread clinical use. There are two schools of thought on the cause of this "reaction". One school,[1-4] while not directly stating that acacia is toxic *per se*, believes that it should not be used because of this toxicity, and the other,[5, 6, 7] that this toxicity is due to faulty preparation or to the presence of some contaminant. The success of various clinics with the intravenous use of acacia lends support to the latter opinion. Meanwhile what the proper method of preparation should be and what contaminant causes this reaction is not known.

PROTOCOL.
Acacia-Glucose—Commercial.
Ampoule No. 1.
No. 22—16.5 kg (Happy), January 14, 1936.

Time	Temp. °F	W.B.C.	Remarks
10:30 A.M.	102.4	16	50 cc intravenously.
10:35			Vomitus, undigested food.
10:45			Depressed.
11:00			Vomitus, mucus.
11:15			Liquid stool, brown.
11:25	103.6	3.2	
11:30			Shivering, urine clear, small amount
12:00	104.8		
12:30 P.M.			Vomitus water, mucus.
1:00	106.4		Shivering
1:05			Light brown, unformed stool, slightly tinged with blood; vomitus bile, mucus; dog very depressed.
2:15	105.8		
3:25	104.3		

W.B.C. = Leucocytes in thousands.

1 Lee, *J. A. M. A.*, 1922, **79**, 726.

2 Hanzlik, De Eds, and Tainter, *Arch. Int. Med.*, 1925, **36**, 447.

3 Bernheim, *J. A. M. A.*, 1919, **73**, 172.

4 Studdiford, *Surg. Gynec. and Obstet.*, 1937, **64**, 772.

5 Bayliss, *J. Pharm. and Exp. Therap.*, 1920, **15**, 29.

6 Keith, Reports of the Special Investigation Committee on Surgical Shock and Allied Conditions, No. 1-8, Special Reprint Series No. 25. Wound Shock and Hemorrhage, Medical Research Committee, Oxford University Press, 285, 1919.

7 Huffman, *J. A. M. A.*, 1929, **93**, 1698.

The following study is an attempt to throw some light on the cause of this toxicity.

The symptomatology of a typical reaction in the dog is exemplified by the protocol. This experiment is one of the 4 reported by Studdiford[4] in which a commercial preparation of gum-glucose which had caused 5 deaths and one marked reaction in the wards, was given to dogs. The amount given was 50 cc. The symptomatic picture is in marked contrast to Exp. 1 in the table in which 500 cc of non-reactive acacia was given.

The similarity of the train of symptoms in an acacia reaction to the "pyrogenic" reaction of reactive infusion fluids,[8] reactive inulin,[9] and the reaction following the intravenous administration of typhoid vaccine,[10] leads to the question whether the acacia "reaction" might also be due to the pyrogen.

Accordingly, non-reactive acacia was inoculated with bacteria known to produce pyrogen, incubated for various periods of time,

TABLE I.
Bacterial Production of Pyrogen in Acacia.*

Organism	Incubation period	Wt of dog (kg)	Volume injected (cc)	Change in temp. (°F)	Changes in W.B.C. (× 1,000)	Symptoms
Control		12	500	100.6-100.0	9.7-9.3	None
B. subtilis (from culture slant)	96 hr	13	100	101.4-103.8	20.9-3.4	Marked shivering
Water organism "B" Genus unidentified (from culture slant)	96 hr	13	100	102.4-104.0	12.5-6.6	None
Water organism "C" Genus unidentified (from culture slant)	1 week at room temp.	12	90	101.0-105.0	25.5-5.7	"
B. subtilis and *Staph. albus* (contaminated by exposure)	48 hr	13.5	450	101.6-104.2	17.7-4.1	Marked shivering

* A sterile commercial 6% acacia in 0.85% NaCl solution was used throughout this series.

8 Co Tui, *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 297; *J. A. M. A.*, 1937, **109**, 250; *Ann. Surgery*, 1937, **106**, 1089.

9 Co Tui, *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 227.

10 Co Tui, *et al.*, *Ibid.*, in press.

filtered through a Berkefeld (W) candle to remove bacterial bodies, and then injected intravenously into the dog. The results in Table I show that the acacia after bacterial growth becomes "reactive".

Conclusions. 1. Acacia *per se* is not "reactive" or pyrogenic. 2. Commercial acacia prepared for intravenous use may be divided into 2 categories, reactive and non-reactive. 3. The growth in acacia of *B. subtilis* and 2 water organisms, all pyrogen-producers, changes non-reactive acacia into reactive acacia. 4. The febrile agent in reactive acacia is probably pyrogen.

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Formation of Peroxide and a Reversible Oxidation-Reduction in Solutions of Sulfanilamide.

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In accordance with a previous concept[1] of the mechanism of sulfhemoglobin formation I investigated whether sulfanilamide which often causes sulfhemoglobinemia forms H_2O_2 in presence of molecular oxygen. Aqueous solutions of sulfanilamide were prepared in different concentrations. The phenolphthalin reagent and, as a catalyst, copper sulfate, both as described by Schales,[2] were added. Oxygen was bubbled through the solutions. Parallel controls contained distilled water instead of sulfanilamide solution. The development of a red color resulting from the oxidation of phenolphthalin into phenolphthalein under the given conditions is specific for the presence, which implies the formation of hydrogen peroxide or labile peroxides. Concentrations of H_2O_2 as low as $1:10^8$ are detectable by means of this method.[2] In solutions containing sulfanilamide in a concentration of 250 mg per 100 cc and more the formation of H_2O_2 was regularly found. In lower concentrations, *e. g.*, 100 mg per 100 cc, no convincing specific effect was detectable.

From the failure to find H_2O_2 in the less concentrated solutions one could conclude that possibly not the sulfanilamide itself but rather

[1] Barkan, G., and Schales, O., *Hoppe-Seyler's Z. f. physiol. Chemie*, 1938, **254**, 241; **253**, 83; 1937, **248**, 96; Barkan, G., "*Kongressbericht II*" *des XVI Internat. Physiol. Kongresses*, Zürich (Schweiz), 1938, 250.

[2] Schales, O., *Berichte Dtsch. Chem. Gesellsch.*, 1938, **71**, 447.