A Preliminary Report . . .

Serum Recovery From the Packing Industry

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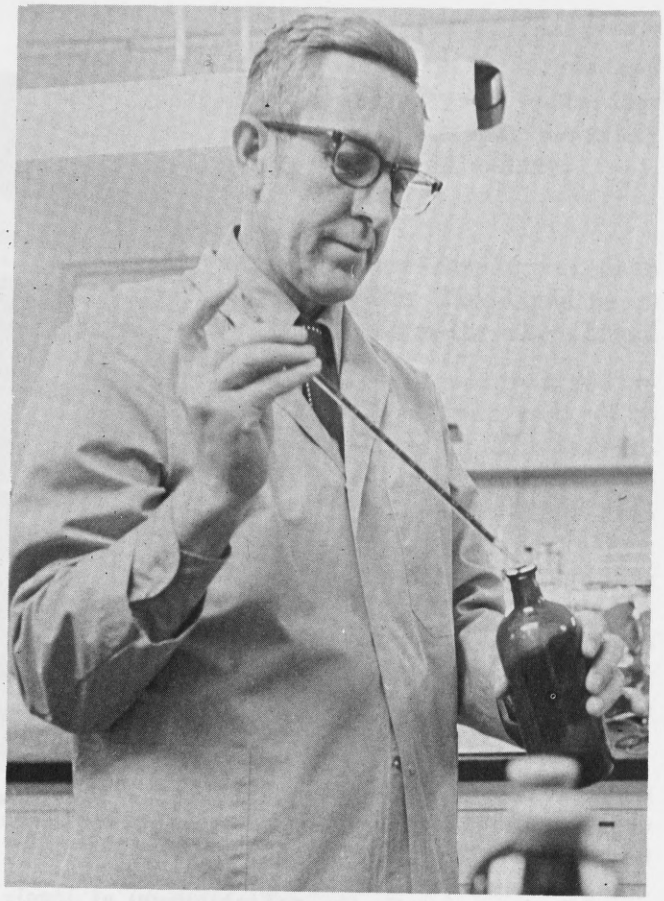

Fig. 1. As one of the first steps in his studies of using blood serum in calfhood disease control, Dr. Staples adds chemical sterilant to a sample of serum from slaughter blood.

Blood and blood components have been used for many years to reduce death losses caused by calfhood diseases (1, 2, 9, 10, 11, 12).

The cliche "too little and too late" could often be applied to the use of blood or serum in calf disease control. Economics is the chief reason these products are not used more extensively. It is costly in veterinary time to collect and administer sterile whole blood, and blood has a short storage life. It is likewise costly to use commercial antiserums in the amounts suggested as necessary to be most effective (1, 4, 12). Such amounts (700 ml or more) would often equal or exceed half the value of the calf being treated.

Substantial reduction in death losses among young calves with diarrhea has been accomplished through the use of ordinary plasma or serum from adult cattle (10, 12). Such products can also substitute for colostrum (10). It has been reported (4) that adult bovine serum is as high as commercial anti-serums in gamma globulin, a major antibody fraction found in blood serum. These and other reports suggest an answer to the cost problem. It might be solved by finding a practical and economical method of recovering serum and plasma from the packing industry where blood is a low-priced or a waste product. Some calves, even after ingesting colostrum, appear to be very short of circulating antibodies and would probably get increased resistance from a generous injection of blood, plasma or serum from adult cattle.

With these and other considerations in mind, investigation of problems related to collecting, sterilizing, storage and using slaughter blood and serum have been initiated. The time-consuming procedures necessary to collect blood aseptically from an automated modern kill line were obviously impractical. An alternate approach was to investigate agents or methods that could render blood or serum sterile without destroying immune properties. Ideally, an agent or method should be viricidal, bacter-

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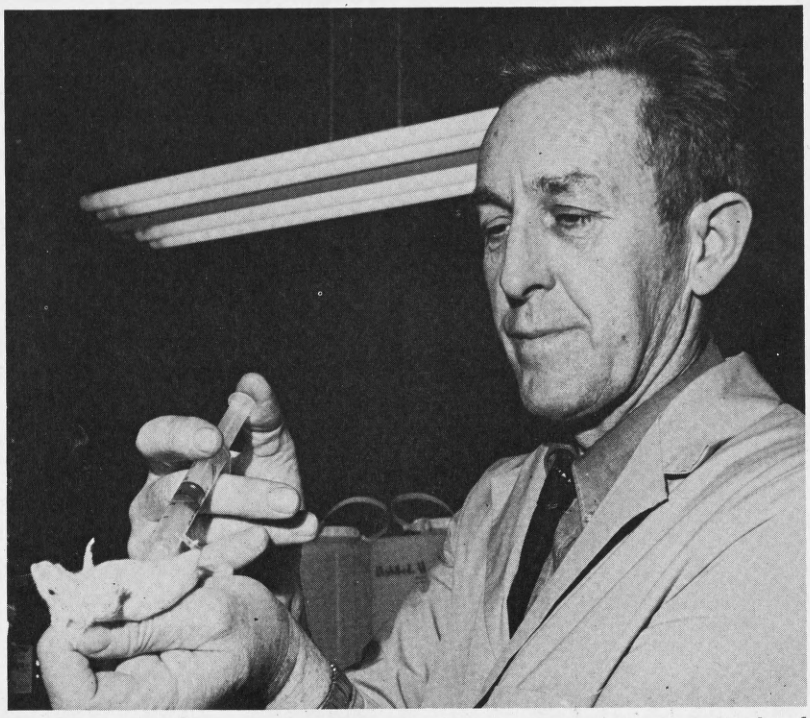

Fig. 2. Dr. Staples gets a measure of the relative toxicity to animals of the various treatments for sterilizing the serum from intraperitoneal injection of the treated serum into laboratory mice.

icidal and mycostatic (fungus-inhibiting) at levels low enough to be relatively nontoxic to calves.

Antibiotics, heat and phenol were initially tried as serum sterilants. Each has disadvantages. Antibiotics are not viricidal or mycostatic. Heat at temperatures required to destroy various microorganisms will cause coagulation and destroys some disease-inhibiting powers in the serum (3, 8). Phenol employed at low levels in aseptically collected commercial serums produced severe reactions in mice, baby pigs and rabbits when injected in large doses. It is also doubtful that such levels would destroy the bacterial loads in serum collected from an automated kill line.

Literature search (5, 6, 7) revealed an interesting and promising compound, beta-propiolactone (BPL). It has been used successfully as a viricide and bactericide in human blood fractions as well as in sterilizing human tissues for surgical transplant. It is a colorless liquid, stable when stored between −15° and −30° C. When mixed with aqueous solutions it is very unstable and rapidly degrades into relatively nontoxic forms, chiefly hydracrylic acid. During the degradation process it will completely inactivate a variety of bacteria and virus. In the pure form it must be handled with the same precautions appropriate in handling strong acids and alkalies.

Procedures

I. Blood Collection and Serum Separation

Blood was collected in sterile gallon jars by the sticker on the kill line of a local packing plant.* One gallon was taken from each animal without slowing the automated process of the line. After clotting and standing overnight at approximately 5° C., the serum was poured off and residual red cells removed by centrifugation employing sterile techniques.

II. Bacterial Cultures

Following centrifugation, standard colony counts were made on serum from each jar to establish bacterial load. Bacterial culture in differential media was performed on 50 lots to establish the genera of bacteria present. No attempt was made to establish the ratio of the different genera in any of the lots containing mixed cultures. Those samples containing three million or more bacteria per ml were designated "high count" and those below this figure designated "low count". Serums so designated were pooled in the appropriate category and a

*Sincere appreciation is expressed to the management and personnel at the Siouxland Dressed Beef Plant (S. E. Needham), West Fargo, North Dakota, for their excellent assistance in collecting blood for experimental use.

final bacterial count made on each of the pooled lots.

Cultures were also made on a number of matching clots, especially where serums were negative to colony counts or to culture of a loop of serum on differential media. The clots were fragmented into sterile physiological saline by means of an improvised "clot breaking tube" and culture made from the solution after vigorous shaking. Clot cultures sometimes yielded one or more genera of bacteria in addition to those isolated from the matching serum. It was also found that it was necessary to incubate a small amount of an apparently negative serum sample overnight and reculture to assure that sterility was absolute. Culturing of a loopful of serum or making a plate colony count would not always disclose growth where the sample was of extremely low bacterial count.

III. Sterilization and Storage

Storage procedures utilized various types of containers including 10-liter polyethylene bags (originally designed for milk-dispensing containers), 500 ml glass serum bottles with rubber stoppers, and a variety of screw-top culture tubes and glass bottles. Glass containers were sterilized by autoclaving. Polyethylene containers depended on the chemical sterilant to achieve sterility during the serum-sterilizing process.

One phase of the study compared the keeping qualities of serum treated with different sterilization methods, then stored under three different temperatures (approximately 5° C, 22° C and incubator temperature of exactly 37° C).

Four methods were used to sterilize serum.

1. Phenol 1:500 (volume to volume).
2. Heat 60° C for 30 minutes.
3. Penicillin — dihydrostreptomycin sulfate at 0.5 percent (volume to volume) with potency of 200,000 units of penicillin G and 250 mg dihydrostreptomycin sulfate per ml of additive.
4. Beta-propiolactone 3 ml per liter of serum. Two products, 97 percent and 99 percent pure were premixed with 4 parts water immediately prior to adding to the serum. In pilot trials, 1 to 1.5 ml BPL per liter was bactericidal for most serum samples.

Sterility checks for bacteria were performed 24 hours following the sterilizing procedure. Some lots were rechecked, particularly the larger ones where serum had been withdrawn for animal injections. No procedures related to virus isolations were performed.

IV. Animal Injections

Serum which was stored in bulk lots and maintained sterility and good physical appearance was utilized in calf disease studies after the safety was tested by injecting laboratory animals.

Results

I. The serum yield varied from 20 percent to 33 percent of the blood volume. Yield could be increased through centrifugation of the whole blood.

II. The bacterial loads in more than 200 individual gallon samples ranged from zero (0) to 19,205,000 bacteria per ml of serum. It is postulated that the negative serums either possessed inhibitory qualities (3, 8) or contained a slight antibiotic residue, since aseptic collection techniques were impractical under the automated conditions of the kill line. Another possibility is that such a sample contained relatively few bacteria and these were trapped in the clot and removed. Checking the matching clot from some negative serum samples yielded bacterial growth from the clot.

Table 1 lists the different genera (more than one species in some genera) of bacteria distinguished and shows the frequency of occurrence in 50 samples representing serum from individual animals before sterilization. Many samples contained two or more genera.

Table 1. Genera of bacteria in 50 blood samples.

Genera	Frequency of Occurrence
Aerobacter	15
Bacillus	3
Candida	1
Clostridium	2
Corynebacterium	2
Diplococci	12
Escherichia	41
Gaffyka	1
Micrococci	19
Pasteurella	1
Proteus	4
Pseudomonas	21
Serratia	13
Staphylococci	58
Streptococci	38

III. Bacterial sterility was achieved in certain lots of serum by all four methods. Beta propiolactone appeared superior when both sterility and physical appearances were considered during long-term storage at environmental temperatures of 5° C and 22° C. None of the methods gave consistently good results regarding physical appearance on

samples stored at 37° C, even though a few samples preserved with BPL appeared to be suitable for intravenous (I.V.) use for up to two years stored at 37° C. Some samples preserved with BPL appear suitable for I.V. use after four years storage at 5° C. Those samples of heat-sterilized serum which remained sterile compare favorably in physical appearance with the BPL-preserved samples after long-term storage. Acceptable physical appearance was based on normal viscosity and absence of particles and precipitates.

Two chief problems were seen in the storage serum besides the fact that few of them maintained good physical appearance after several months storage at 37° C. One problem was slow-developing mycotic forms. The other was precipitation of particles that would render the serum unsuitable for I.V. injections. Possible solutions to these problems might be to include an additional mycostatic agent prior to sterilization. Adjusting the pH toward neutral might assist in reducing precipitates since treatment with BPL lowered the pH approximately 0.5 point for every ml of the compound added per liter of serum. Filtering the serum after several months storage at 5° C produced good physical appearance for extended periods.

IV. Routine sterility checking after sterilization procedures had been applied was used to establish a safety index prior to injecting serum in calf disease studies. Mice were the usual animals used since they proved to be relatively unaffected by heterologous serum injections as compared to rabbits. Injecting five ml of sterilized serum via intraperitoneal route into 40 gram (approximately) mice for three consecutive days produced less than 25 percent mortality except in the case of serums sterilized with phenol. Four such consecutive injections of commercially prepared serums (which are apparently prepared with phenol) produced 89 per cent mortality (in 55 mice). The same dosage of serum sterilized with 3 ml per liter of BPL produced 26.3 per cent mortality in 51 mice.

In calves, serums having high bacterial loads prior to sterilization appeared to be more difficult to administer intravenously or intraperitoneally without causing undesirable clinical symptoms such as rapid and labored breathing and signs of discomfort and depression. Other factors also appear to be involved, as certain low bacterial count serums from any of the four methods of sterilization also showed undesirable symptoms especially when administered intravenously. Such undesirable reactions may be due to pH readings of 5.0 to 6.0 or possibly to different protein fraction patterns.

These possibilities require further investigation. Little difference could be seen in clinical signs when sterilized serums from "high count" bacterial lots and "low count" bacterial lots were given orally as a colostrum substitute. Up to 1,000 ml of BPL-sterilized serum were administered via the intraperitoneal route within a 15-minute period without the animals showing more than a transient discomfort. Observable signs and symptoms when injected into calves or piglets indicate serums sterilized with beta-propiolactone are at least as safe as commercial preparations. A major problem in using the most promising agent tried (beta-propiolactone) is to clear the compound with the Food and Drug Administration for use in food-producing animals.

Summary

Initial investigations of the practicality of obtaining bovine serum from packing house operations have shown promising results. Of the four sterilizing procedures used, the most promising is the use of the chemical agent beta-propiolactone as a sterilant. It appears to have good bactericidal and viricidal properties (the latter indicated from literature review) (5, 6, 7) as well as fair mycostatic properties. It also showed low toxicity at effective sterilization levels when serum so sterilized was injected into calves, piglets or mice.

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