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# THE EFFECTS OF DIETHYLENE GLYCOL MONOBUTYL ETHER ON THE BACTERIAL POPULATION OF FRESHLY FLAYED CALFSKINS

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## ABSTRACT

When freshly flayed calfskin was tumbled in a 100 percent float of a 20 percent aqueous butyl Carbitol solution, there was shown to be an initial 80 percent reduction of the bacterial population present in the contact solution when compared to the bacterial population present when water alone was used. The bacterial population in a sample of the 24-hour supernatant was shown to remain low after an additional 24 hours of tumbling and during an extended room temperature storage period of five days.

Gram stain tests were made on selected bacterial colonies during plate count studies and all the colonies tested proved to be gram positive bacilli.

The treatment of freshly flayed calfskins with a 20 percent by weight aqueous butyl Carbitol solution has been previously shown to result in a skin which air dries white and clean. The added feature of this treatment to decrease and control bacterial population in the treating supernatant should complement its usefulness in hide preservation applications.



## INTRODUCTION

The use of aqueous solutions of alkylene glycols and their derivatives for the dehydration of hides and skins and an evaluation of this dehydrated stock as a substrate for leather have been reported in previous publications (1, 2). Freshly flayed calfskins were used in the course of this work. Such skins are naturally contaminated with manure, body filth, blood, and microorganisms. This report describes the treatment of such skins with an aqueous solution of diethylene glycol monobutyl ether (butyl Carbitol®†) and its effect on the bacterial population.

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†Reference to a commercial product does not constitute recommendation by the U. S. Department of Agriculture over any other similar products not mentioned.

## EXPERIMENTAL METHODS AND DISCUSSION OF RESULTS

Three freshly flayed calfskins were obtained at an abattoir. They were put in plastic bags, packed in ice, and transported back to our Laboratory. The skins were trimmed and cleaned of any large pieces of flesh and fat. They were then treated with a 100 percent float of a 20 percent by weight butyl Carbitol ( $C_4H_9O[C_2H_4O]_2H$ ) solution in water by tumbling at six r.p.m. for 24 hours in a stainless steel lined drum. The skins were then toggled and allowed to air dry.

There was no objectionable odor associated with the skins or supernatant after treatment and the skins were visibly clean and free of any noticeable microbial growth, such as slimes or color formers. The skins dried white and clean and the hair was tight.

The first determination of a bacterial count was carried out on the aqueous butyl Carbitol solution that had been used to treat the three freshly flayed calfskins for 24 hours. A test sample of this supernatant was poured into a sterile tube. The supernatant was brown and a microscopic examination showed large amounts of irregularly shaped insoluble material. A plate count was made using DIFCO Plate Count Agar as the medium. The bacterial population on the 24-hour supernatant was 3500 bacteria per ml. The remaining test sample was then refrigerated.

Another experiment was carried out on this supernatant to determine if these bacteria might become acclimated to the solution with a resultant increase in bacterial population. The supernatant test solution which had been refrigerated for six days was allowed to come to room temperature. It was then sampled for bacterial count after holding for one, two, and five days at room temperature. The bacterial counts were 5000, 4400, and 4050 bacteria per ml., respectively. There was no evidence of increased growth and it was evident from these results that butyl Carbitol was exerting a desirable effect in keeping the bacterial population at a low level.

At this point it was decided to add two percent butyl Carbitol to the plate count agar to determine if this might be needed in the medium to stimulate the growth of these bacteria which have been exposed to high concentrations of butyl Carbitol. The butyl Carbitol was added to the plate count agar before it was sterilized. The bacterial count on the five-day supernatant using this medium was 2900 bacteria per ml., compared to 4050 bacteria per ml. on the normal medium. The data indicated that the presence of two percent butyl Carbitol in the plate count medium did not stimulate an increase in bacterial population over the control.

A screening test was carried out on the five-day sample to estimate the spore-forming bacteria present. A test sample was heated to 80°C. and held at this temperature for 20 minutes. Bacteria which survived this treatment were pre-

sumably spore-formers (3). Plate counts were made on the heated sample using both plain plate count agar and plate count agar that had two percent butyl Carbitol added to it before sterilizing. The results were as follows: before heating, 4050 bacteria per ml.; after heating and without butyl Carbitol present, 654 bacteria per ml.; after heating and with butyl Carbitol present, 44 bacteria per ml. These data indicated that about 15 percent of this bacterial population were presumably spore-formers. However, it was interesting to note that when two percent butyl Carbitol was present in the plate count medium, the population of spore-formers was reduced by more than 90 percent. This indicated that the heat treatment rendered the spore-forming bacteria more sensitive to a killing effect or to a germination-inhibiting effect by the plate count agar containing the butyl Carbitol.

Gram stains were made on smears taken from selected colonies in the course of the plate count studies. All the colonies tested were found to be gram positive bacilli.

To gather some data on the effect of the treatment on the bacterial population at zero time and 24 hours, 100-gram pieces of freshly flayed calfskin were treated in 100 percent floats of water alone and in 20 percent by weight solutions of butyl Carbitol in water. Plate counts were made after 15 minutes of vigorous agitation and after 24 hours of gentle agitation. The 15 minutes of vigorous shaking was to help wash the bacteria free from the skin and hair and to break up any bacterial clumps present. This was then considered to be the zero time sample.

The bacterial counts were as follows: without butyl Carbitol present, the zero time count was 1,120,000 bacteria/ml. and after 24 hours the count increased to 640,000,000 bacteria/ml.; with butyl Carbitol present, the zero time count was 200,000 bacteria/ml. and after 24 hours it decreased to 98,000 bacteria/ml.

At zero time the presence of butyl Carbitol in the float water gave an approximate 80 percent reduction in bacterial population when compared to the counts in float water alone. This is evidence of a bactericidal effect by the butyl Carbitol. After 24 hours the bacterial count in water alone had increased 570 times, while the presence of the butyl Carbitol in the water had resulted in a 50 percent decrease in the bacterial population.

We have also noted the ability of the butyl Carbitol to inhibit mold growth. This might be expected, however, because it has been reported that all the glycols inhibit mold growth (4).

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