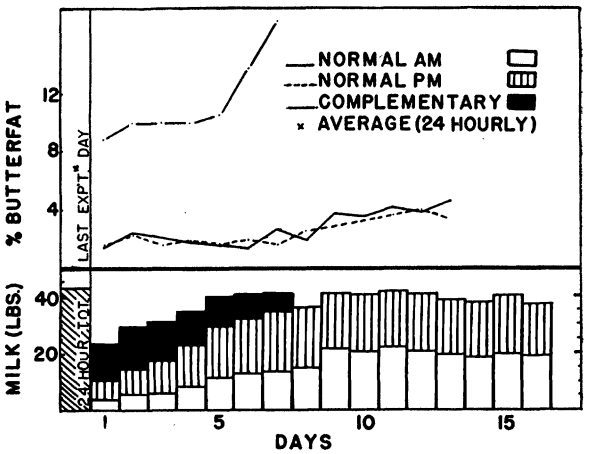


FIG. 1. Daily milk production in the recovery period following 156 hours of hourly milking. Twenty international units of oxytocin had been administered at each hourly milking. The total milk and average butterfat percentage of only the last 24 hr is indicated previous to the recovery period. Complementary milk was removed using decreasing amounts of oxytocin for a period of seven days in the recovery period at which time there was evidence of complete recovery of the ability to eject milk efficiently.

regular milking. At this second milking the condition was little improved. At this time she produced 6.5 lb of milk with a butterfat percentage of 1.6. The complementary milk was removed with the aid of an intravenous injection of 20 iu of oxytocin. There were 13.0 lb of this milk with a butterfat percentage of 8.9. The same procedure was followed in each of the next seven days. Oxytocin was given once daily in decreasing amounts according to the following schedule: 20, 20, 10, 6, 6, 3, 3 iu.

The complete recovery period data is presented in Fig. 1. The daily milk is partitioned into normally obtained A.M. and P.M. milks and P.M. complementary milk. The butterfat (%) of each of these fractions is also indicated.

In the days following the first day it is seen that the portion of milk which is complementary was decreasing in amount and increasing in butterfat content. The normally obtained amount of milk increased regularly throughout this week. At the seventh day, the complementary milk was considered normal (*5, 6*) and it was decided that she could go it alone. Subsequent production of milk and fat was essentially in the range of what might be expected. During a 10-day period following the third day after the last oxytocin injection she produced at a daily rate of 33.0 lb with a butterfat test of 3.9%.

This is believed to be the first reported instance in which treatment using exogenous oxytocin had brought about a refractoriness to the usual let-down stimuli in a normally responsive animal. It is logical to postulate that this blockage occurred at one of two sites. Either the posterior pituitary gland output of oxytocic substance was suppressed by the large amount of exogenous oxytocin that had been administered or the alveolar myoepithelial elements of the mammary gland had lost their sensitivity to physiological amounts of endogenously released oxytocic substance. The large amount of oxytocin administered over the previous 6½ days (3120 iu) appeared to disturb physiological relationships between the posterior pituitary gland and the contractile elements surrounding the alveoli of the mammary gland, but there is no evidence to favor which end of this axis had been blocked.

Although it is considered remote, there was the possibility that the hourly injection schedule may have prompted a conditioned reflex release of amounts of epinephrin sufficient to block the normal response to endogenously released oxytocic substance but not sufficient to block the effect of the administered hormone. One would then have to assume that the same conditions which had developed the conditioned release of epinephrin would not be maintained after changing the frequency of stimulation even after the reflex had become strongly established. It is possible that milk let-down blocking epinephrin may have been released as the result of a painful stimulus due to the high intramammary pressure occasioned by the change in milking frequency. The return to normal in the cow then being an adjustment to the reestablishment of this increased volume in the udder.

This paper records the observation that the milk ejection mechanism of a cow which was normally responsive was severely deranged. The most plausible explanation of this phenomenon is advanced.

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## Paper Chromatography of Chlorophylls[1]

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In the hope of avoiding decomposition of chlorophylls reported to occur when they are subjected to paper chromatography, we impregnated the paper with sucrose and found that the degree of decomposition was brought virtually to zero. At the same time about the same degree and order of resolution into separate components was obtained as with the sucrose column and thus a mixture of chlorophylls

[1] Research carried out under the auspices of the U.S. Atomic Energy Commission.

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*a* and *b* was separated into two distinct spots and likewise a mixture of chlorophylls *b* and *b'*. In our experiments the developer consisted of ½% *n*-propyl alcohol in *n*-hexane, a solution which has proved effective in the analyses of chlorophylls with sucrose columns.[3]

*Impregnation of the Paper.*[4] (1) The impregnating solution consisted of 0.18 g/ml solution sucrose (Jack Frost sugar 6X) in distilled water, filtered through a Büchner funnel. Approximately this concentration seemed important for obtaining separations. (2) Strips of Whatman 1 chromatographic paper 3 cm wide were thoroughly wetted by soaking in this solution. (3) The wet papers were hung for drying in an oven at 100° C.

*The Ascending Chromatogram.* The next two steps were carried out in a semidarkened room. (1) The spot or spots to be analyzed were placed along a line across the paper about 3 cm from the bottom and were dried under a stream of nitrogen gas. (2) The strip was hung in a 250-ml graduated cylinder into which 10 ml developer had been added. Nitrogen gas was swept through to displace the air from the cylinder which was then stoppered. (3) The cylinder was placed in a refrigerator where the spots could develop at about 5° C in the dark.

[3] We are indebted to Miss LaVelma Thompson, now of the Rohm and Haas Co., and Kenneth Owens, Chemistry Department, University of Minnesota, for recommending this developer to us.

[4] Kirchner, J. G., and Keller, G. J. *J. Am. Chem. Soc.* **72**, 1867 (1950), included sugar among the substances with which paper may be impregnated for chromatography.

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## The Use of a Fraction of Mechanically Disrupted Cells for Production of Group A Streptococcus Typing Antisera

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The production of diagnostic antisera for the typing of Group A streptococci depends on the development of antibodies against the type-specific M proteins. Since these proteins are poorly antigenic in the purified state (*1*), the standard procedure for the production of streptococcus typing antisera (*2*) involves rabbit immunization with whole cell vaccines. Serum so produced must be absorbed to remove group-specific and other non-type-specific antibodies, a procedure which frequently results in sufficient loss of type-specific titer to render the serum useless. The work described here was undertaken in an attempt to reduce or eliminate the need for absorption through the use of vaccines containing the M proteins with only enough additional cellular material to render them adequately antigenic.

The approach was suggested by the report of Harris (*3*) on sonic disintegration of streptococci. In the present study streptococci were disrupted by mechanical means (*4*) and vaccines prepared from the fraction stated by Harris to contain most of the M protein. A method was developed which has proved to be of considerable value.

The packed cells from 500 ml of an 18-hr Todd-Hewitt broth culture of streptococci are suspended in 10–15 ml of physiological saline solution and transferred to a vaccine bottle (50–60 ml capacity) which is one-third full of sterile glow beads.[1] The bottle is closed with a sterile rubber stopper secured with adhesive tape, rolled in cotton, packed tightly in a paint can, and clamped in a paint shaker. After 1½ hr of shaking a gram-stained preparation of the suspension is examined. If more than occasional intact streptococcus cells are seen, the material is shaken another ½–1 hr. Most strains of streptococci are disintegrated in 1½ hr; a few require 2–3 hr.

When satisfactory disintegration has been effected, the fluid is poured off, the glow beads are washed with 40–50 ml of physiological saline, and the washings added to the first material removed from the bottle. The opalescent fluid so obtained is spun in a Servall centrifuge for 30 min at 5000 rpm. The supernatant fluid is discarded and the sediment is taken up in physiological saline equal in amount to one-fourth the volume of the original broth culture. The vaccine is heated for 30 min at 60° C to kill any cells which may have survived the shaking process. When the sterility check is completed, the vaccine is ready for use. The same immunization schedule is followed as that recommended by Lancefield for standard vaccines.

Vaccines made with this technique have been used successfully to produce antisera for 16 types of Group A streptococci. They failed to give good antisera for 3 types (34, 35, 44), 2 of which also failed with standard vaccines. Although simultaneous parallel studies with glow bead and standard vaccines have not been done, it is felt that results have been sufficiently clear-cut to warrant a report, with comments on the following facts and observations:

Antisera have been produced with these vaccines against 11 types (5, 6, 11, 13, 17, 19, 22, 36, 38, 40, and 42) for which it is relatively easy to prepare sera with standard vaccines, and also against 5 types (2, 8, 25, 27, and 32) for which it is notoriously difficult to prepare sera.

Antisera obtained from animals immunized with glow bead vaccines have little or no antibody for the group-specific carbohydrate (C substance). An absorption ratio of 20 parts of serum to one part of packed streptococcus cells usually is sufficient to remove group-specific and nonspecific cross-reacting antibodies. This is in contrast to the ratio of 3–6 volumes of serum per volume of packed cells required for absorption of sera produced with standard vaccines. It is probable that these absorption ratios are

[1] Cataphote No. 12 Glass Beads, Cataphote Co., Toledo, Ohio.