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.

Manuscript received October 12, 1953.

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.

largely a reflection of the amount of C substance in the vaccines. Standard vaccines subjected to the acid-heat extraction procedure yield extracts which give strong reactions with both type-specific and group-specific antisera. On the other hand, glow bead vaccines similarly treated yield extracts which give strong type-specific reactions but weak or negative group reactions. Furthermore, if samples of the supernatant fluids (discarded in the preparation of the vaccines) are tested with standard Group A antiserum, they give strongly positive reactions, indicating that these supernates contain large amounts of C substance.

It appears that the disruption of the streptococcus cells and subsequent differential centrifugation serve to separate most of the group-specific C carbohydrate from the type-specific M protein. At the same time sufficient cellular material is retained in the sediment to render the M protein antigenic.

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Manuscript received October 8, 1953.

Sulfhydryl and Disulfide in Keratinization[1]

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The common concept of sulfur metabolism in epidermal keratinization has been that the sulfhydryl groups of the Malpighian layer are converted to the disulfide bridges of the keratin molecule (*1–4*). This concept, based largely on histochemical evidence, is not fully supported by evidence derived from modern histochemical sulfhydryl reagents (*5, 6*). Direct chemical determinations of —SH and —S—S— in cutaneous structures are fragmentary, a circumstance which prompted the present study.

Sulfhydryl in ether-washed powdered tissues was measured with the specific Bennett reagent (*7*) after incubating the tissues in water for 1 hr at 40° C or in a 10% solution of Duponol (sodium lauryl sulfate) for 10 min in a boiling water bath. The increase of reactive —SH groups by the latter treatment is not due to splitting of —S—S— linkages inasmuch as: prolonged identical treatment of cystine or insulin yielded no —SH groups; keratin pretreated with iodoacetate, washed repeatedly with water, and then incubated with Duponol, yielded no —SH groups; lithium bromide, a hydrogen bond breaker (*8*), had the same effect as Duponol of increasing the —SH values of keratin.

Disulfide was estimated after conversion to sulfhydryl by incubating the tissues in a boiling water bath with 2 *M* KCN in 0.1 *N* NaOH. With this procedure, the disulfide values of insulin (*9*) and of various horny structures (*10*) were in agreement with data of previous investigators.

Human epidermis was isolated from surgical and autopsy specimens by the stretch method (*11*). Frozen plantar skin was cut into successive horizontal sections 50–75 μ thick; a portion of each section was examined microscopically to determine its histologic nature.

TABLE 1. Sulfhydryl and disulfide content of various skin structures.*

Tissue	Aqueous —SH†	Duponol —SH‡	% Sulfur as —S—S—	% Sulfur as —SH + —S—S—
Man				
Hair	6	11	5.7	5.7
Nails	9	146	3.41	3.46
Plantar calluses	14	49	0.342	0.358
Cow				
Hoof (lateral)	67	230	5.4	5.5
Hoof (plantar)	11	34	2.20	2.21
Hoof (Malpighian layer)	—	57	0.360	0.378
Horn (deep layers)	—	229	4.09	4.16
Horn (surface layers)	41	112	1.67	1.71
Horse				
Burr (horny layer)	9	82	0.305	0.331
Burr (Malpighian layer)	224	590	0.098	0.287

* Sulfhydryl expressed as mM × 10^{-2}/100 g dry weight tissue.
† Values obtained after incubating tissues in water for 1 hr at 40° C.
‡ Values obtained after incubating in 10% Duponol for 10 min in a boiling water bath.

TABLE 2. Sulfhydryl and disulfide content of human skin.*

Tissue	Aqueous —SH†	Duponol —SH‡	% Sulfur as —S—S—	% Sulfur as —SH + —S—S—
Total plantar epidermis	25	88	0.341	0.369
Layers of plantar skin:				
Keratin	13.5	51	0.334	0.345
Keratin	24.5	65	0.323	0.343
Keratin + Malpighian layer	26	115	0.353	0.390
Malpighian layer + small amt. keratin	49	153	0.361	0.410
Malpighian layer + small amt. corium	—	305	0.317	0.415
Corium	0	7	0.033	0.035

* Sulfhydryl expressed as mM × 10^{-2}/100 g dry weight tissue.
† Values obtained after incubating tissues in water for 1 hr at 40° C.
‡ Values obtained after incubating in 10% Duponol for 10 min in a boiling water bath.

[1] This study was supported by U.S.P.H.S. Grant C-3601.
[2] National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, Bethesda, Md.