tionately more ducklings following when their posthatch age was more than 17 hours ($\chi^2 = 6.71$, $p < .01$). Analyses of the strength of following in the young and older ducklings (Table 1) indicate that the older birds also followed the silent model more *strongly* than the younger ones (Mann-Whitney Tests, $p < .02$ in both age base lines).

As can be seen further in Fig. 1, Fig. 2, and Table 1, vocal stimulation exerted a very potent effect on the instigation and the strength of following at all ages in both baselines. The statistical information concerning the increase in incidence of following addressed to the vocal model over the silent one follows: posthatch age 8 to 17 hours ($\chi^2 = 10.9$, $p < .001$) and 18 to 50 hours ($\chi^2 = 7.67$, $p = .005$); developmental age 27 days, 0 to 11 hours ($\chi^2 = 9.9$, $p = .002$), 27 days, 12 to 23 hours ($\chi^2 = 5.33$, $p = .02$), and 28 days, 0 to 11 hours ($\chi^2 = 5.57$, $p = .02$). The statistical analyses concerning the increase in strength of following the vocal model over the silent one are consistent with the above results and are presented in Table 1.

The present evidence indicates that the duckling's ability to follow a model sheerly on the basis of visual stimulation increases with age and that auditory stimulation plays an important activating or attention-directing function at all ages. These findings are consonant with the implications of the previous work outlined in the introduction of this report. In addition, the present laboratory demonstration of the enhancing effect of auditory stimulation coordinates well with the naturalistic observation (*6*) that auditory stimulation is a prominent component of "imprinting" as it occurs in the wild (*7*). At present there is no evidence to suggest that tactile, thermal, or olfactory stimulation play a significant role in instigating the following-response (*8*).

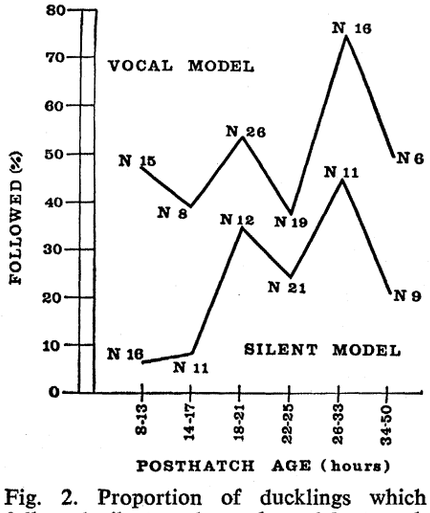

Fig. 2. Proportion of ducklings which followed silent and vocal model at each posthatch age range.

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4. The apparatus has been described (*2*). From the standpoint of visual perception processes, it should be noted that the model appears at all points in the apparatus against a relatively homogeneous, "flat black" background from the ducklings' angle of regard. The apparatus also differs from that of other investigators in that it is not of the circular maze type—that is, in the present apparatus the model can be seen by the duckling at any point in the field should the duckling attend to the model.
5. Physical and other details of this call have been described by G. Gottlieb [*J. Comp. Physiol. Psychol.* **56**, 86 (1963)].
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7. Without benefit of detailed knowledge of the naturalistic situation, a number of researchers have implicitly or explicitly acknowledged the enhancing value of sound in instigating the following-response. The earliest reports were by: D. A. Spalding, *Macmillan's Magazine* **27**, 282 (1873); K. Lorenz, *J. Ornithol.* **80**, 50 (1935); E. Fabricius, *Proc. 10th Intern. Ornithol. Congr.* (1950), p. 375; A. O. Ramsay, *Auk* **68**, 1 (1951).
8. Research supported by funds from the North Carolina Hospitals Board of Control and U.S. Public Health Service grant M-6039. The technical assistance of Carter Doran, Anne Smith, Frank Lane, and Linda Marsh Green is gratefully acknowledged.

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Biosynthesis of Streptococcal Cell Walls: A Rhamnose Polysaccharide

Abstract. *Protoplast membranes from* Streptococcus pyogenes *incorporated rhamnose into a preexisting polysaccharide when incubated with thymidine diphosphate rhamnose-C*^{14}. *This polysaccharide, when extracted from the membranes, did not give a precipitin reaction with group A antisera, but could be coprecipitated with added group A polysaccharide by acetone. It is presumed to be a precursor to group specific polysaccharide of the streptococcal cell wall.*

The group-specific antigen, or C-substance, of the cell walls of *Streptococcus pyogenes* is a polysaccharide. L-Rhamnose and *N*-acetyl-D-glucosamine have been identified as the only carbohydrate constituents of the C-polysaccharide (*1*) of the group A organism. A methylation study has shown that the glycosidic bonds between the rhamnose units involve carbon atoms 1 and 3. Side chains are attached at carbon atom 2 (*2*). Some of the *N*-acetylglucosamine is linked terminally and is responsible for the serologic response to antiserum to the group A streptococci (*2*, *3*).

L-Rhamnose of the streptococcal cell walls is derived solely from D-glucose without scission or rearrangement of the glucose carbon skeleton (*4*). This conversion occurs through thymidine diphosphate sugar intermediates in other organisms (*5*). The same pathway occurs in group A streptococci.

We now describe an enzyme system in protoplast membranes of *S. pyogenes*, type 14, strain S_{23}, which transfers rhamnose from thymidine diphosphate L-rhamnose (TDP-Rh) to a rhamnose-containing polysaccharide present on the membranes. This particulate enzyme system is obtained from lysed protoplasts by the use of a purified lysin (*6*). That the enzyme preparation consists largely if not exclusively of protoplast membranes is deduced from the microscopic appearance, the absence of nucleic acids, the high lipid content, and the behavior with detergents. The TDP-Rh-C^{14} was prepared from glucose-C^{14}-1-phosphate (*5*) and contained 11 percent TDP-D-glucose-C^{14}, from which it could not be readily separated. In a typical experiment 40 to 50 percent of the radioactivity added as TDP-Rh-C^{14} was recovered in a polysaccharide extracted from the membranes after incubation (Table 1).

The product of the enzymic reaction

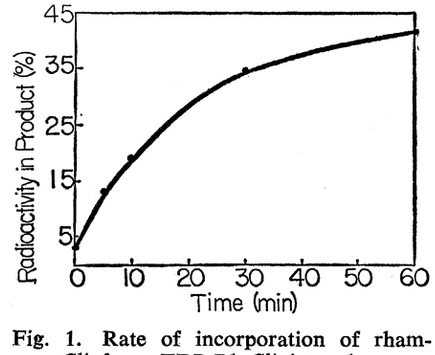

Fig. 1. Rate of incorporation of rhamnose-C^{14} from TDP-Rh-C^{14} into rhamnose polymer of protoplast membranes.

Table 1. Incorporation of radioactivity from TDP-Rh-C^{14} and UDP-NAcGm into polysaccharide of protoplast membranes.

System	Radioactivity		Radioactivity in polysaccharide (%)
	Source	Amount (count/min)	
Complete*	TDP-Rh-C^{14}	12,000	44
Less UDP-NAcGm	TDP-Rh-C^{14}	12,000	50.5
Boiled enzyme	TDP-Rh-C^{14}	12,000	0.06
Complete	UDP-NAcGm-H^3	2,500	5.4

* Complete system: 0.05 μmole TDP-Rh; 0.01 μmole UDP-NAcGm; 0.1*M* phosphate *p*H 7.4; 0.005*M* mercaptoethanol; 0.02*M* $MgCl_2$; 13.5 mg enzyme protein in final volume of 1 ml. Incubated 60 min at 37°C.

was made soluble by treating the thoroughly washed membrane particles with 5 percent trichloroacetic acid at 90°C. The product is chromatographically immobile; on acid hydrolysis, the only radioactive substance produced was rhamnose, recognized by paper chromatography. The polysaccharide product probably has a molecular weight greater than 4000, since it is excluded from a column of Sephadex G-25 gel. Acetone precipitation of the product in the presence of added group A polysaccharide, followed by redissolving and reprecipitation with acetone, gave essentially constant specific activity in the two precipitates (124 and 117 count/min per milligram of rhamnose, respectively).

Microscopically the protoplast membrane preparations showed only an occasional form which might be an unlysed protoplast or possibly a whole cell. Confirmation that the enzymic activity was attributable to membranes and not to the presence of any residual whole cells was obtained, however, by incubating TDP-Rh-C^{14} and uridine diphosphate *N*-acetylglucosamine (UDP-NAcGm) with whole cells equal to one-tenth the amount used for preparing the protoplast membranes for a comparable incubation. Less than 2 percent of the added radioactivity was reisolated in the trichloroacetic acid fraction from the incubated whole cells.

The rate of polysaccharide formation was determined by removing samples from an incubation and determining the amount of polysaccharide formed and the amount of TDP-Rh which had disappeared. The samples were extracted with 5 percent trichloroacetic acid and the extracts were fractionated on a column of Sephadex G-25 gel. Eighty percent of the maximal incorporation of radioactivity occurred within the first 30 minutes of incubation (Fig. 1).

Since the omission of UDP-NAcGm (Table 1) did not reduce the incorporation of rhamnose into the polysaccharide, tritiated UDP-NAcGm was substituted as source of radioactivity. Only 5 percent of the radioactivity was detected in the trichloroacetic acid extract (Table 1).

We are dealing, therefore, with the addition of rhamnose units to a preexisting rhamnose polymer in cytoplasmic membranes that is quite low in hexosamine (compared with C-polysaccharide in the cell wall) and does not serve as a good acceptor of additional acetylglucosamine from UDP-NAcGm. This observation is in agreement with the lack of serologic reactivity as measured by precipitin formation of the membrane polymer with group A antibody. However, the existence of the polymer, tightly bound to membranes which have the enzymatic capacity for its augmentation, suggests a precursor relationship to the C-polysaccharide of the cell walls. It may be that an additional branching enzyme prepares rhamnose side chains to which a transferase for UDP-NAcGM attaches acetylglucosamine moieties as end groups (see *2, 3*). It may also be that unbranched chains of rhamnose polymer are synthesized in the membranes and transferred to the cell wall; also the subsequent modification of these or the addition of acetylglucosamine moieties or both, may be performed on wall polymers by enzymes existing in the membranes.

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* CIBA fellow in microbiology.

13 February 1963

Neutron Activation Analysis of Fluid Inclusions for Copper, Manganese, and Zinc

Abstract. *Microgram quantities of copper, manganese, and zinc, corresponding to concentrations greater than 100 parts per million, were found in milligram quantities of primary inclusion fluid extracted from samples of quartz and fluorite from two types of ore deposits. The results indicate that neutron activation is a useful analytical method for studying the content of heavy metal in fluid inclusions.*

Studies of microscopic fluid inclusions that occur in many minerals (*1, 2*) suggest that in some cases the trapped fluids are identical with the fluid from which the host mineral was precipitated. Such inclusions, termed "primary," have received particular attention from economic geologists interested in the chemical environment and mechanisms of ore deposition. Determinations of the major ionic constituents of these fluids have been feasible for a number of years (*1–3*).

Of special interest is the heavy (ore) metal content of primary inclusions, for it may be related to the concentration of heavy metals in the ore-forming fluid. Estimates of the concentration of the metal elements in these ore-forming fluids have ranged from less than 1 part per million (ppm), in saline brines, to that of a metallic sulfide melt. A recent indirect estimate (*4*) sets limits of 1 to 1000 ppm on the concentration range that might reasonably be expected in the fluids which formed many ore deposits.

Difficulties in the procedures of extraction and analysis have precluded valid determinations of even the order of magnitude of the heavy metal content of inclusion fluids up to the present time, but recent developments in technique (*5*) permit the extraction of relatively uncontaminated inclusion fluid from very small samples. We now de-