more helpful than morphine in preventing post-operative intestinal stasis.[6] The isolated gut shows no qualitative differences in reaction to morphine and dilaudid, and a morphinized gut shows no further depression with dilaudid and vice versa. In general, the findings of Uhlmann and Abelin[7] were confirmed in regard to the relative effects of various concentrations of morphine. While these observations are merely indicative of possible effects in patients, it may be expected that dilaudid will have much the same qualitative action on the gut as morphine but probably with less intensity in equivalent therapeutic dosage.

7797 C

Transformation of Hemolytic Streptococci.*

THOMAS REICH. (Introduced by W. H. Manwaring.)

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Two methods of typing hemolytic streptococci have been suggested by recent investigators: (a) the Lancefield method[1] based on the existence of at least 5 antigenically distinct type-specific "carbohydrates" in different human, veterinary and environmental strains, and (b) the Tillett-Garner-Madison technic,[2] based on the presence of at least 3 different type-specific fibrinolysins. Both methods assume that the selected diagnostic character is genetically stable.

To test this assumed stability an attempt was made to transform a typical antihuman fibrinolytic strain of *S. hemolyticus* into a non-fibrinolytic strain of apparent veterinary origin. The strain selected for this attempt was originally isolated by Lancefield from a case of scarlet fever. The strain (C203) is specifically lytic for human fibrin and contains only one type-specific carbohydrate.

To make the proposed transformation, 4 rabbits were injected intraperitoneally with doses varying from 2 to 20 cc. of a 24-hour

[6] Orr, T. G., *Ann. Surg.*, 1933, **98**, 835.

[7] Uhlmann, F., and Abelin, R., *Z. exp. Path. Therap.*, 1920, **21**, 58.

*Supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

[2] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485. Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 641.

beef-heart-infusion broth culture. Three days later, the rabbit receiving 20 cc. was in a moribund condition. Blood was withdrawn by cardiac puncture and plated out on 2% rabbit-blood beef-heart-infusion agar. A single typical hemolytic colony on this plate, was used for the production of the broth culture for inoculation of the second group of 4 rabbits. This process was repeated for 22 successful animal passages.

Three times, during the 18 months this work was in progress, the virulence of the strain increased to approximately 700 times that of the original broth culture. In each case, however, the acquired virulence was almost completely lost before the next animal passage.

At varying stages of the transformation the recovered hemolytic colony was retitrated for the 5 Lancefield type-specific carbohydrates and for the Tillett-Garner antihuman fibrinolytic enzyme. Typical data thus obtained are recorded in Table I.

TABLE I. Effects of Repeated Rabbit Passage on Fibrinolytic Streptococci.

Five type-specific antistreptococcus serums were prepared from rabbits immunized against formalized type-cultures kindly furnished by Dr. Lancefield. Each antiserum gives specific precipitin reactions with 1:128 dilutions of the homologous Lancefield carbohydrate, and no demonstrable reaction with heterologous streptococcus extracts, the technic throughout being identical with that used by Lancefield.

In recording the data: ++++ represents precipitin reactions in dilutions as high as 1:128, +++ 1:32, ++ 1:8, and +1:2. (Type-specific antiserums B and D gave 0-reactions throughout the experiment.)

The antihuman fibrinolytic titres were determined by the Tillett-Garner plasma-clot technic; ++++ representing complete liquefaction of the normal human plasma-clot within 20 minutes after mixture with the 24-hour broth culture, +++ within 2 hours, ++ 6 hours, + 24 hours, and 0 no demonstrable softening by the end of 24 hours.

No. animal passages	"Carbohydrate" titer			Fibrinolytic titer
	Type A	Type C	Type E	
C203 before animal passage	++++	0	0	++++
After 1st rabbit passage	+++±	0	0	++++
" 4th " "	+++	0	0	
" 8th " "	++±	+++	0	+++
" 15th " "	0	0	+++	0
" 22nd " "	0	0	+++	0

From this table it is seen that both the Lancefield Type-A carbohydrate and the Tillett-Garner antihuman thrombolytic function disappeared quantitatively between the eighth and fifteenth animal passage. Two Lancefield carbohydrates were demonstrable in the eighth recovery strain. Both gave place to a third Lancefield carbohydrate (E) by the fifteenth animal passage. By this time, therefore, the original antihuman fibrinolytic strain of *S. hemolyticus*

had been successfully transformed and stabilized as a non-fibrinolytic strain of presumptive bovine origin.

That this is a justifiable interpretation of our data is shown by the almost perfect reversion of the 15th recovery strain to its original antihuman diagnostic specificities by the 7th subculture on routine culture medium. Typical stages in this reversion are recorded in Table II.

TABLE II. Qualitative and Quantitative Reversion on Routine Culture Medium.

A single hemolytic colony recovered from the 15th rabbit passage was transplanted at 2-week intervals on routine 2% rabbit-blood beef-heart-infusion agar. Titrations as in Table I.

No. of subculture	"Carbohydrate" titer			Fibrinolytic titer
	Type A	Type C	Type E	
C203 before animal passage	++++	0	0	++++
C203 after 15th rabbit passage	0	0	+++	0
3rd subculture of recovered strain	0	0	0	++
5th " " " "	++	0	0	+++
7th " " " "	+++±	0	0	+++
10th " " " "	++++	0	0	+++
15th " " " "	++++	0	0	+++±

7798 C

Susceptibility of "Hybrid" Fibrins to Streptococcus Fibrinolysins.*

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Tillett and Garner[1] found that both of the human-rabbit heterologous fibrinogen-thrombin complexes are liquefied by the specific antihuman fibrinolysin formed or secreted by certain strains of *S. hemolyticus,* native rabbit fibrin being refractory to this specific bacterial lysin.

Quantitative differences, however, are demonstrable between the susceptibilities of the 2 Tillett-Garner hybrid fibrins. Fibrin formed by coagulating human-fibrinogen with rabbit-thrombin, for example, may require a 1 :48 concentration of a given streptococcus filtrate to show demonstrable lysis. One-and-a-half times this lytic dose, or a

* Supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.