All animals in Series B possessed actively secreting mammary glands. The extent of this development as far as the size of the gland is concerned was greatest in the males, somewhat less in the ovariectomized females, and least in the non-castrate females. A white, fatty fluid (milk?) flowed freely from cut portions of these glands. Histological examination showed a flattened epithelium, prominent vacuolization and alveoli distended with fluid containing many fat droplets and numerous free vacuolated cells.

The effect of long continued injections of estrin upon the mammary glands and hypophysis suggest that the hypophysis is functioning abnormally. Whether this lactation is the result of estrin administration *per se* or whether it resulted after the cessation of the injections is not known, as the animals were sacrificed 4 days after the last injections. This question is now being investigated.

7579 C

Fibrinolytic Activity of Hemolytic Streptococci on Blood of Cases of Recurrent Tropical Lymphangitis.

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Tillett and Garner[1] have recently shown that broth cultures of hemolytic streptococci of human origin rapidly dissolve normal human fibrin clot. Tillett, Edwards and Garner[2] demonstrated the development of resistance to dissolution in the plasma clot obtained from individuals following acute hemolytic streptococcus infections. They also showed that this antifibrinolytic property is absent in the fibrin clot derived from a group of patients convalescing from other infections. Likewise, the blood from the great majority of healthy adults and from persons with other acute diseases was found to be susceptible to fibrinolysis. The authors believe that this insusceptibility to dissolution is specifically induced and that "the fibrinolysin of hemolytic streptococci, in the body, makes a definite response directed against the lytic action of the bacteria."

While studying the probable relationship of hemolytic strepto-

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

[2] Tillett, W. S., Edwards, L. B., and Garner, R. L., *J. Clin. Invest.*, 1934, **12**, 47.

cocci to recurrent tropical lymphangitis, we have made a series of fibrinolytic tests with the blood of a group of patients suffering from this condition. In performing the test the method employed was that recommended by Tillett, Edwards and Garner[2] with the exception that we used tryptic digest broth instead of glucose broth to grow the streptococci.

Thirty-three strains of hemolytic streptococci isolated from different conditions were tested for their fibrinolytic activity on the plasma of a normal individual. All determinations were made at the same time, by the same person, and under similar conditions. The results are shown in Table I.

TABLE I.

Fibrinolytic Activity of Strains of Hemolytic Streptococci from Different Sources.

Strain No.	Source	Complete dissolution in hrs.
*N.Y.$_5$	Scarlet fever	None†
M_4	Sore throat	4 hr.
S_2	Septicemia	8 "
*T	Tonsils	2 "
M_5	Sore throat	5/6 "
S_1	Septicemia	3½ "
T_5	Tonsils	3½ "
O_1	Osteomyelitis	2¼ "
T_1	Tonsils	12 "
T_5	"	2½ "
A_{10}	Meninges	2½ "
A_8	Abscess	5/6 "
A_3	Lesion on knee joint	2½ "
A_4	Pustule	4 "
T_6	Tonsils	5 "
T_3	"	8 "
M_2	Sore throat	20 "
T_8	Tonsils	2/3 "
T_{10}	"	2½ "
A_5	Deep chronic lesion on face	1¼ "
T_4	Tonsils	10 "
T_{11}	"	4 "
A_{11}	Pustule	2 "
*E	Erysipelas	.2/3 "
L_{14}	Lymphangitis	1 "
L_6	"	1 "
L_{12}	"	1¼ "
L_5	"	5 "
A_{12}	Impetigo lesion on leg	3 "
L_7	Lymphangitis	3½ "
L_9	"	Partial dissolution in 24 hr.
L_{11}	"	2 hr.
L_8	"	4½ "

*Kindly sent to us by Dr. A. F. Coburn.
†None in 24 hrs.

From these, 2 strains, Nos. T_8 and E, were selected for carrying out the fibrinolytic determinations. The results are shown in Table II.

TABLE II.
Fibrinolytic Determinations.

Case	Condition		Strains	
			E_1	T_8
			Complete dissolution in	
A.P.	Normal		1 hr.	2 hr.
M.E.M.	"		2/3 "	2/3 "
L.G.	"		1¼ "	2/3 "
E.R.	"		1 "	2/3 "
J.M.	"		2/3 "	2 "
M.N.	"		½ "	2/3 "
T.V.	"		2 "	2/3 "
G.V.	Typhoid	Convalescent	3½ "	1 2/3 "
C.A.	"	3rd week	1 2/3 "	1 2/3 "
A.S.	"	" "	Clot retraction	1½ "
A.C.	"	1st "	None†	7 "
N.C.	Thrombophlebitis	Acute attack	2½ hr.	1¼ "
J.G.	Common cold	5 days after attack	2 "	1 2/3 "
Z.C.	R. lymphangitis	2 mo. after attack	12 "	5 "
R.A.	Lymphangitis	Attack subsiding	None†	None†
J.L.	R. lymphangitis	24 hr. after onset	None†	None†
M.R.	" "	2 " " "	14 hr.	8 hr.
P.L.	" "	24 " " "	None†	None†
M.R.	" "	1 " " "	6½ hr.	22 hr.
P.L.	Lymphangitis	2 days " "	None†	None†
M.R.	R. lymphangitis	24 hr. " "	None†	None†
F.J.	" "	3 days " "	None†	None†
J.C.*	" "	4 " " "	2/3 hr.	2/3 "
J.P.*	" "	6 " " "	½ "	½ "
F.M.	R. lymphadenitis and lymphangitis	9 " " "	None†	None†

*In these 2 cases, pure cultures of hemolytic streptococci were isolated from local lesions at the time determinations reported were made.
†None in 24 hrs.

Repeated determinations at varying intervals were made in several cases of recurrent tropical lymphangitis. In 4 cases the plasma clot exhibited maximum resistance from the onset of the acute attack to 6 weeks after the attack. In one case, the dissolution time was 14 hours, 2 hours after the onset of symptoms, and maximum resistance on the 18th and 59th day after the attack. Another case exhibited maximum resistance when the attack was subsiding and showed complete dissolution in 10 hours, 8 days after the attack; and in 8 hours, 24 days after the attack.

In 2 cases in which virulent hemolytic streptococci were isolated from local lesions in the affected limb during the acute attack, fibrinolysis was complete in 30 minutes during the attack, and in 2 hours, 8 days after the attack.

In a normal control, where repeated determinations were made at short intervals during 2 months, the dissolution time varied slightly from 30 minutes, the lowest, to 1 hour and 30 minutes, the highest.

Summary: Fibrinolytic determinations made with 33 strains of hemolytic streptococci isolated from different conditions showed individual variations in their lytic activity when tested under similar conditions. Apparently, the plasma clot derived from cases of recurrent tropical lymphangitis develops a definite resistance to the fibrinolytic activity of hemolytic streptococci.

7580 C

Refinements in X-ray Technique for the Estimation of Vitamin D.*

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None of the existing techniques commonly employed for the estimation of Vitamin D are entirely beyond criticism. In choosing the most suitable procedure a number of factors must be considered, depending upon whether time and economy or accuracy and reliability are to receive the most consideration. To indicate briefly some of these factors, we have but to point out the principal advantages and limitations of the 3 widely used methods for the assay of antirachitic potency, in all of which inbred stocks of albino rats furnish the experimental animals.

Since all 3 methods may be adapted to either curative or preventive procedures and since the former enjoy by far the greater vogue in this country, the present discussion and experimental work are limited to curative methods. The Steenbock diet No. 2965 was used to produce rickets, and, in general, the recommendations of the Committee of the American Drug Manufacturers Association on Vitamin Assay were followed.[3]

The bone-ash technique as worked out principally by Chick, Roscoe and others[1, 2] is a purely objective procedure, not subject to aberrations of human judgment, even to the extent that they occur in the other 2 methods. On the other hand, the factor of biological variation exerts its greatest influence in this method, and the statis-

* We wish to express our thanks to Dr. Stafford L. Warren and the Department of Radiology of Strong Memorial Hospital for suggestions and the use of X-ray equipment and also to the Department of Biochemistry for animal facilities.

[1] Chick and Roscoe, *Biochem. J.*, 1926, **20**, 137.

[2] Chick, Korenchevsky, and Roscoe, *Biochem. J.*, 1926, **20**, 622.

[3] Holmes, *Rep. A. D. M. A., Com. Vit. Assay*, 1932.