mine whether the difference in fibrinolytic activity is qualitative or quantitative and whether a relation exists between the apparently constant production of anticoagulant in glucose-broth by hemolytic enterococci and the final low pH of the broth, characteristic of the *S. fecalis* group.

The anticoagulant of enterococci is not necessarily an artificial product occurring *in vitro* only. Recently it was shown[15] that the *Enterococcus hemolyticus* anticoagulant may occur *in vivo:* the fluid obtained from the lesion in a case of Ludwig's angina, caused by hemolytic enterococci showed anticoagulating properties as did the isolated strain when cultured in glucose-broth. Meanwhile, we found bacterial anticoagulants in 17 other exudates of various origin such as abscesses, empyemic fluid, etc. In 5 of them the culture revealed enterococci, which were found in pure culture in one case and in the remaining 4 cases together with *B. coli, B. welchii* and staphylococcus respectively. It cannot yet be decided whether the anticoagulating properties of such exudates are due to one factor or to different factors and whether the anticoagulant produced *in vivo* is identical with that *in vitro*. The demonstration of bacterial anticoagulants in natural infections supplements the recent finding of *S. hemolyticus* fibrinolysin in human lesions.[16]

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Chick Embryo Broth and Chick Embryos, Held in Cold Storage, as Sources of Growth Stimulants for Tissue Cultures.

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It is frequently difficult during the winter months in certain localities to obtain the adequate and inexpensive supply of fertile eggs so essential where constant supplies of embryo juice are required for procedures in tissue culture. Two methods were devised for utilizing chick embryos from which growth-promoting extracts might be made while a plentiful supply of embryos was available.

Embryo Broth. Five hundred grams of finely chopped embryos

15 Neter, E., and Young, G. S., *Am. J. Dis. Child.*, 1937, **53**, 1531; Neter, E., *Arch. Path.*, 1937, **23**, 295.

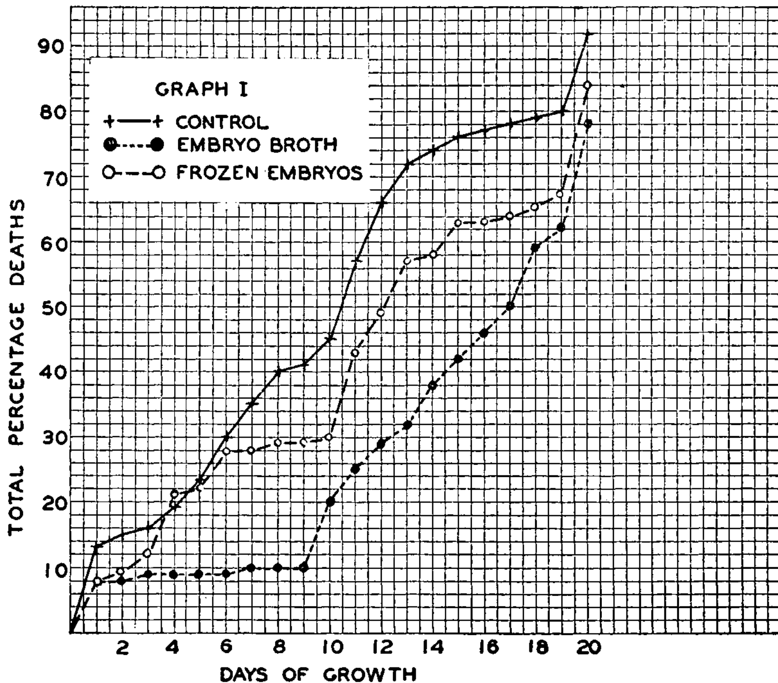
16 Neter, E., and Witebsky, E., *J. Bact.*, 1936, **31**, 77; Neter, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 735.

were steeped in one litre of Tyrode's solution for 24 hours in a refrigerator and then heated for 2 hours in an Arnold steam sterilizer. The resulting broth was strained through fine cheese cloth and as much fluid as possible forced from the cooked tissue. Ten grams of peptone and 5 gm. of sodium chloride were added, the whole filtered through paper and made up to one litre with Tyrode's solution and sterilized in an Arnold steamer for 30 minutes. This fluid was the concentrated stock solution. Portions of it were diluted with Tyrode's solution to make a 2% solution, adjusted to pH 7.6, sterilized in flasks in an Arnold steamer for 2 hours and stored at 30°F. in a refrigerator. The final fluid was used in lieu of fresh embryo juice.

Frozen Embryos. Eight-day chicks were removed aseptically from their shell and placed in sterile section dishes—2 or more to a dish. These were placed in a larger, sterile, glass-covered container and stored at 10°F. in a refrigerator. From these frozen embryos, 25% embryo juice could be made in the usual manner at any time.

The broth and frozen embryos were kept in storage, as described, for 6 months and were thereafter tested for growth-promoting substances against embryo juice made from fresh 8-day embryos.

The cultures for the entire experiment were made by the cover-



slip-hanging-drop method. Aseptic precautions were observed during all manipulations and all water used was triply distilled in a pyrex glass apparatus. The plasma was obtained by centrifuging blood drawn, without the use of an anticoagulant, from the wing veins of young hens. The embryo juice (25%) for the control cultures was prepared by extracting eight-day chick embryos in Tyrode's solution (pH 7.5 to 7.6), containing dextrose.

For the first test series with the media, 129 cultures of 8-day chick heart were planted in equal parts of plasma and the prepared broth; thus, making a 1% concentration of broth in the planting medium. For the second test series, 145 cultures of heart tissue from 8-day chick embryos were planted, in the same manner, substituting for the broth, a 25% solution of embryo juice prepared from the frozen embryos. Control cultures, 180 in number, were prepared at the same time, using the same heart tissue, the same plasma and 25% embryo juice made from fresh 8-day embryos. All the cultures were incubated at 37°C. and a daily record of each was kept for 20 days. From the data collected on the control and from the 2 experimental series, average percentage death rate curves were constructed (Graph 1). With the use of a delineascope and planimeter the area of the original explant was measured

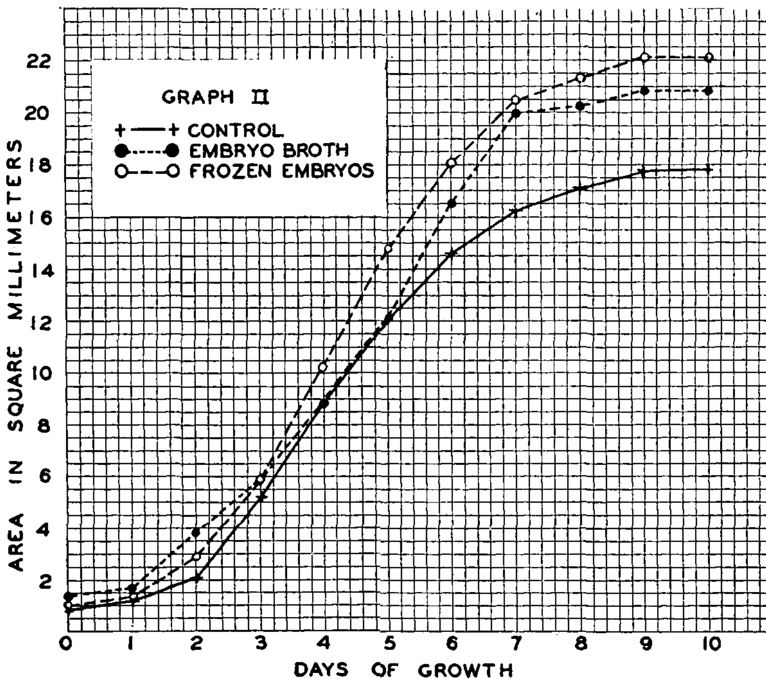


TABLE I.

No. of Days	Control			Frozen Embryos			1% Broth			Frozen Embryos vs. Control				1% Broth vs. Control			
	Means	Probable Error	Standard Deviation	Means	Probable Error	Standard Deviation	Means	Probable Error	Standard Deviation	σ_D*	D_M	$3\sigma_D$	Significant Difference	σ_D*	D_M	$3\sigma_D$	Significant Difference
O.T.	0.89	±.03	0.40	0.97	±.02	0.39	1.37	±.04	0.61	0.54	0.08	1.62	0	0.07	0.48	0.21	+
1	1.16	±.03	0.46	1.31	±.03	0.50	1.69	±.05	0.76	0.66	0.15	1.98	0	0.89	0.53	2.67	0
2	2.07	±.11	1.64	2.96	±.10	1.64	3.97	±.13	2.11	0.23	0.89	0.69	+	0.26	1.90	0.78	+
3	5.16	±.22	3.31	5.88	±.15	2.28	5.93	±.22	3.46	0.77	0.72	2.31	0	0.47	0.77	1.41	0
4	8.89	±.30	5.00	10.14	±.27	4.12	8.92	±.28	4.44	0.63	1.25	1.89	0	0.65	0.03	1.95	0
5	12.04	±.43	6.57	14.71	±.38	5.83	12.05	±.26	5.32	0.85	2.67	2.55	+	0.82	0.01	2.46	0
6	14.55	±.58	8.19	18.01	±.41	6.32	16.51	±.44	6.80	1.01	3.46	3.03	+	1.03	1.96	3.09	0
7	16.22	±.72	10.86	20.51	±.47	7.07	20.00	±.48	7.48	1.27	4.29	3.81	+	1.30	3.78	3.90	0
8	17.03	±.62	9.38	21.29	±.49	7.42	20.34	±.46	7.14	1.17	4.26	3.51	+	1.15	3.31	3.45	0
9	17.73	±.39	6.00	22.07	±.51	7.68	20.89	±.44	6.86	0.95	4.34	2.85	+	0.88	3.16	2.64	+
10	17.73	±.39	6.00	22.07	±.51	7.68	20.89	±.44	6.89	0.95	4.34	2.85	+	0.88	3.16	2.64	+

*σ_D, Standard Error of Difference of Means; D_M, Difference of Means; Significant Difference, $D > 3\sigma_D$.

and the daily outgrowth of each culture was recorded for a period of 10 days. At the close of the experiment, 109 cultures planted with broth; 105 planted with extract from frozen embryos; and 103 controls utilizing fresh embryo juice, had lived for 10 days or longer. These cultures form the basis for the results recorded in Table I and Graph 2.

Microscopical study of each of the cultures in the experimental series showed no marked differences or variations in the behavior of the cells or any structural changes which would differentiate these cells from those in the control series. Many dividing cells were observed and an abundant outgrowth took place in all the cultures. Graphs 1 and 2 are self-explanatory. Examination of Table I indicates that increased growth of tissue cultured in 1% chick embryo-broth, is not sufficiently greater than that of tissue grown in fresh embryo juice to make the former medium of any particular value except as a matter of convenience during periods when fresh embryos are scarce. On the other hand the difference in growth of cultures utilizing embryo juice made from frozen embryos compared with the growth of cultures in juice from fresh embryos, is so much greater as to be of real significance. Furthermore, the use of juice made from frozen embryos precludes the possible contamination of cultures due to the introduction of viable cells from insufficiently centrifuged fresh extracts.

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The Biuret and Ninhydrin Tests for Proteins as Measured with Hardy's Spectrophotometer.

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Color tests for proteins have long been in use, although in most cases the chemistry involved is not clearly understood. One method of attack upon this problem is to analyse the transmission spectra of the colored solutions with the aid of Hardy's recording photoelectric spectrophotometer. This has been done for the ninhydrin and biuret reactions.

*Contribution No. 107 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Mass.