

ACTION OF SERUM ON FIBROBLASTS IN VITRO.

By ALEXIS CARREL, M.D., AND ALBERT H. EBELING, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

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I.

INTRODUCTION.

It had been observed previously that fragments of connective tissue, cultivated in plasma, did not increase in mass in spite of the activity they displayed in their medium. The growth of fibroblasts was no more extensive in serum than in media containing a small amount of serum or no serum at all, even when embryonic tissue juice was added to the medium.¹ Under the conditions of the experiments, serum never increased cell activity. As the action of serum had been determined merely by the rate of cell migration during a short time,¹ it was necessary to ascertain whether the duration of life of pure cultures of fibroblasts was modified by the presence of serum in varied dilutions and by its omission.

II.

EXPERIMENTAL.

The tissues were obtained from a 10 year old strain of fibroblasts,² and the serum from the plasma of young adult chickens which had fasted for 48 hours. The fibroblasts were cultivated in a medium of fibrinogen suspension,³ Tyrode solution, and in varied amounts of serum. The medium was modified in different ways by substituting for Tyrode solution the requisite amounts of serum or tissue juice, according to the nature of the experiments. In order to prevent

¹ Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 317.

² Ebeling, A. H., *J. Exp. Med.*, 1922, xxxv, 755.

³ Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiii, 641.

the digestion of the fibrin in a serum-free medium, a small amount of egg yolk was added. Coagulation of the fibrinogen suspension was brought about by embryonic tissue juice in a concentration of 1:200. The composition of the medium was as follows:

Fibrinogen suspension 1 volume.
 Doubly concentrated Tyrode solution containing 1 per cent
 egg yolk 1 volume.
 Tyrode solution containing 1:50 embryonic tissue juice 1 volume.
 Tyrode solution 1 volume.

TABLE I.

Influence of 0 Per Cent and 7 Per Cent Serum on the Duration of Life of Homologous Fibroblasts.

Passage No.	Culture No.	Experiment 1.			Experiment 2.			Experiment 3.			Experiment 4.		
		Relative increase.		Ratio: $\frac{S}{T}$.	Relative increase.		Ratio: $\frac{S}{T}$.	Relative increase.		Ratio: $\frac{S}{T}$.	Relative increase.		Ratio: $\frac{S}{T}$.
		Tyrode solution.	Serum.		Tyrode solution.	Serum.		Tyrode solution.	Serum.		Tyrode solution.	Serum.	
1	340	3.38	3.28	0.97	3.53	3.38	0.96	3.93	4.06	1.03	3.67	3.46	0.94
2	351	2.12	2.62	1.23	2.74	2.33	0.85	2.33	2.37	1.02	2.27	2.23	0.98
3	366	1.59	1.60	1.00	2.13	1.78	0.84	2.23	2.04	0.92	1.90	1.83	0.96
4	376	1.40	1.50	1.07	0	1.85		0	1.80	0	0	1.34	
5	386	1.00	1.12	1.12		0.85			0			0	
6	398	0	0.47	0		0							

Passage No.	Culture No.	Experiment 5.			Experiment 6.			Experiment 7.			Experiment 8.		
		Relative increase.		Ratio: $\frac{S}{T}$.	Relative increase.		Ratio: $\frac{S}{T}$.	Relative increase.		Ratio: $\frac{S}{T}$.	Relative increase.		Ratio: $\frac{S}{T}$.
		Tyrode solution.	Serum.		Tyrode solution.	Serum.		Tyrode solution.	Serum.		Tyrode solution.	Serum.	
1	325	3.62	3.80	1.05	4.21	4.21	1.00	3.25	3.69	1.13	3.60	3.00	0.83
2	339	3.48	3.55	1.02	3.29	3.50	1.06	3.03	3.13	1.03	2.69	2.82	1.05
3	350	2.13	2.21	1.04	2.00	2.00	1.00	1.54	1.58	1.03	1.69	1.48	0.87
4	365	1.89	1.66	0.88	1.81	1.72	0.95	1.73	1.62	0.94	1.78	1.62	0.91
5	375	1.70	1.50	0.88	1.60	1.00	0.63	0	0.53	0	2.00	1.85	0.93
6	385	0	0	0	1.50	0	0				1.50	1.30	0.87
7											0	0	0

The medium was modified by adding serum or tissue juice to replace a similar amount of Tyrode solution. The pH of Tyrode solution was 8.0, that of serum and tissue juice from 7.0 to 7.6, and that of fibrinogen suspension 6.8.

TABLE II.
Influence of 0 Per Cent and 25 Per Cent Serum on the Duration of Life of Homologous Fibroblasts.

Passage No.	Culture No.	Experiment 1.			Experiment 2.			Experiment 3.			Experiment 4.		
		Relative increase.		Ratio: $\frac{S}{T}$.	Relative increase.		Ratio: $\frac{S}{T}$.	Relative increase.		Ratio: $\frac{S}{T}$.	Relative increase.		Ratio: $\frac{S}{T}$.
		Tyrode solution.	Serum.		Tyrode solution.	Serum.		Tyrode solution.	Serum.		Tyrode solution.	Serum.	
1	381	3.30	3.35	1.01	2.95	3.05	1.03	2.80	3.00	1.07	2.66	3.20	1.20
2	392	2.55	2.06	0.81	2.60	2.15	0.82	2.45	1.38	0.56	2.20	1.84	0.84
3	402	1.60	1.57	0.98	2.50	1.77	0.70	1.17	0	0	0.78	0.50	0.64
4	409	1.20	0.30	0.25	1.22	0.50	0.41	0			1.80	0.29	0.16
5	415	1.74	0	0	1.53	0	0				0	0	0
6	424	0			0								

Passage No.	Culture No.	Experiment 5.			Experiment 6.			Experiment 7.		
		Relative increase.		Ratio: $\frac{S}{T}$.	Relative increase.		Ratio: $\frac{S}{T}$.	Relative increase.		Ratio: $\frac{S}{T}$.
		Tyrode solution.	Serum.		Tyrode solution.	Serum.		Tyrode solution.	Serum.	
1	388	2.75	2.46	0.90	2.82	2.34	0.83	3.00	3.25	1.08
2	397	1.83	2.10	1.15	2.50	2.56	1.02	2.03	2.30	1.13
3	404	1.80	0.67	0.37	1.40	1.50	1.07	1.57	1.57	1.00
4	410	1.72	0	0	1.68	0.44	0.26	1.24	0.22	0.18
5	416	0			1.58	0.41	0.26	0.90	0.20	0.22
6	423				0	0	0	0	0	0

The tissues were incubated for 48 hours, and the rate of growth was measured in the ordinary manner.⁴ Then they were transferred to a new medium of the same composition and the operation was repeated until death occurred, or until the rates of growth of fibroblasts in

⁴ Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 231.

both experimental and control media had been compared during a number of passages.

1. *Duration of Life of Fibroblasts in Media Containing 0 Per Cent and 7 Per Cent Serum.*—Two groups of four experiments each were made. The results are averaged in Table I. The fragments of tissue in 0 per cent and 7 per cent serum grew at about the same rate. The fibroblasts migrated actively into the medium after each transfer. Nevertheless, the mass of the tissues never increased. After five or six passages, death occurred (Table I).

2. *Duration of Life of Fibroblasts in Media Containing 0 Per Cent and 25 Per Cent Serum.*—The first group was composed of four and the second of three experiments (Table II). The duration of life of the fibroblasts was slightly shorter in 25 per cent serum than in 0 per cent.

3. *Duration of Life of Fibroblasts in Media Containing 50 Per Cent Serum.*—The duration of life of fibroblasts in 50 per cent serum was compared to that in 25 per cent. In 25 per cent serum, death occurred after the fifth or sixth passage; in 50 per cent serum, after the fifth passage (Table III).

4. *Rate of Growth of Fibroblasts in Media Containing 0 Per Cent and 10 Per Cent Serum in the Presence of 25 Per Cent Embryonic Tissue Juice.*—The mass of the tissues increased, and the cultures had to be divided. But the rate of growth was no greater in 10 per cent serum than in the media containing no serum (Table IV). As a rule, the migration of the cells was slightly more active in the serum-free medium. As the life of fibroblasts in media containing embryonic tissue juice may be very long, and possibly indefinite, the condition of the tissues in both media was compared after ten passages. The average ratio of the rate of growth of fibroblasts in 0 per cent and 10 per cent serum was 0.96. There was, then, no difference between the tissues cultivated in media containing 0 per cent and 10 per cent serum, in the presence of embryonic tissue juice.

5. *Rate of Growth of Fibroblasts in Media Containing 0 Per Cent and 25 Per Cent Serum in the Presence of 25 Per Cent Embryonic Tissue Juice.*—The experiments summarized in Table V indicate that the presence of 25 per cent serum failed to increase the rate of growth of fibroblasts.

TABLE III.

Influence of 25 Per Cent and 50 Per Cent Serum on the Duration of Life of Homologous Fibroblasts.

Passage No.	Culture No.	Experiment 1.			Experiment 2.			Experiment 3.		
		Relative increase.		Ratio: 50 per cent serum. / 25 per cent	Relative increase.		Ratio: 50 per cent serum. / 25 per cent	Relative increase.		Ratio: 50 per cent serum. / 25 per cent
		25 per cent serum.	50 per cent serum.		25 per cent serum.	50 per cent serum.		25 per cent serum.	50 per cent serum.	
1	311	4.00	3.00	0.75	3.54	2.54	0.72	4.09	3.00	0.73
2	326	3.24	2.44	0.75	2.78	2.08	0.75	3.06	2.29	0.75
3	338	3.07	2.29	0.75	2.38	1.70	0.71	2.70	1.86	0.69
4	349	1.67	1.06	0.63	1.05	0.68	0.65	1.55	0.89	0.57
5	364	1.63	0.37	0.23	1.18	0	0	1.21	0.36	0.30
6	374	0.64	0		0			0	0	0

TABLE IV.

Influence of 0 Per Cent and 10 Per Cent Serum in the Presence of 25 Per Cent Tissue Juice on the Rate of Growth of Homologous Fibroblasts.

Passage No.	Culture No.	Experiment 1.			Experiment 2.			Experiment 3.			Experiment 4.		
		Relative increase.		Ratio: S / T.	Relative increase.		Ratio: S / T.	Relative increase.		Ratio: S / T.	Relative increase.		Ratio: S / T.
		Tyrode solution.	Serum.		Tyrode solution.	Serum.		Tyrode solution.	Serum.		Tyrode solution.	Serum.	
1	370	4.15	4.00	0.96	3.29	3.44	1.05	3.47	2.73	0.79	3.70	3.49	0.94
2	377	3.36	3.65	1.09	2.93	3.23	1.10	2.90	3.48	1.20	3.25	3.40	1.05
3	387	2.60	2.20	0.85	3.15	2.72	0.86	2.66	2.90	1.09	2.44	2.96	1.21
4	396	1.77	1.87	1.06	1.70	2.30	1.35	1.80	2.15	1.19	2.17	2.42	1.12
5	403	2.17	1.70	0.78	1.83	1.59	0.87	1.60	1.69	1.06	1.75	1.40	0.80
6	408	3.20	2.03	0.63	4.13	2.58	0.62	3.44	3.08	0.90	4.73	3.08	0.65
7	414	6.68	4.92	0.74	4.74	4.85	1.02	5.24	5.80	1.11	5.30	4.15	0.78
8	425	7.78	6.43	0.83	4.70	4.67	0.99	4.73	4.46	0.94	5.66	5.08	0.90
9	429	4.80	4.50	0.94	4.65	4.30	0.92	3.83	4.16	1.09	3.50	3.46	0.99
10	432	3.37	3.45	1.02	4.25	4.30	1.01	3.89	3.89	1.00	4.04	3.62	0.90

III.

DISCUSSION.

The duration of life of fibroblasts was found to be practically identical in media containing no serum and 7 per cent serum. It was slightly shortened by the addition of 25 per cent serum, and still more so by 50 per cent serum. Under the conditions of the experiments, serum proteins were apparently not utilized by the fibroblasts. These results confirmed the information obtained from previous experiments¹ in which the influence of serum on the activity of fibroblasts

TABLE V.

Influence of 0 Per Cent and 25 Per Cent Serum in the Presence of 25 Per Cent Tissue Juice on the Rate of Growth of Homologous Fibroblasts.

Passage No.	Culture No.	Experiment 1.			Experiment 2.			Experiment 3.		
		Relative increase.		Ratio: $\frac{S}{T}$.	Relative increase.		Ratio: $\frac{S}{T}$.	Relative increase.		Ratio: $\frac{S}{T}$.
		Tyrode solution.	Serum.		Tyrode solution.	Serum.		Tyrode solution.	Serum.	
1	433	3.10	3.24	1.05	3.14	3.27	1.04	3.82	3.54	0.93
2	444	4.23	4.50	1.06	4.40	4.52	1.03	4.00	4.30	1.08
3	454	3.46	3.25	0.94	3.35	3.34	1.00	4.32	3.65	0.84
4	466	3.45	2.90	0.84	3.85	3.85	1.00	4.50	4.05	0.90
5*	482	1.41	1.50	1.06	1.74	1.83	1.05	No measurement.		
6	491	3.54	3.43	0.97	4.40	4.35	0.99	4.56	4.56	1.00
7	498	4.15	3.80	0.92	4.15	4.28	1.03	4.15	4.63	1.12
8	513	2.95	3.10	1.05	No measurement.			No measurement.		
9	530	3.14	3.20	1.02	4.10	4.04	0.99	3.39	3.61	1.06

* Cultures divided, one-half discarded. Tracings made after 24 hours growth.

was studied during a short time. After ten passages in the presence of embryonic tissue juice, fibroblasts were as active in Tyrode solution alone as in Tyrode solution containing a small amount of serum. Their rate of growth was slightly decreased when serum was present in a higher concentration.

Serum, therefore, cannot be considered a culture medium in the proper sense of the word. No new protoplasm is built up by the cells from substances contained in the medium. The residual activity,

manifested by the cells in media composed only of plasma, serum, or Tyrode solution, is due to substances stored in the tissues or the cells themselves. The failure of the fibroblasts *in vitro* to utilize the serum proteins confirms the observation made by several experimenters⁵ that tissues cultivated in plasma do not increase in mass, and that cell multiplication may be due to a mere transfer of food material from the central part of the tissue fragment to the periphery. As soon as embryonic tissue juice is added to the medium, the mass of the tissues increases rapidly. But the presence of serum under low or high concentration does not cause the production of a larger amount of tissue. The new protoplasm is made from substances contained in the embryonic tissue juice and not in the serum. Serum proteins are not used by the fibroblasts as food material. They appear merely to decrease the rate of cell multiplication.

IV.

CONCLUSIONS.

It may be concluded that, under the conditions of the experiments:

1. The duration of life of fibroblasts is not altered by the presence of 7 per cent serum in a medium composed of fibrin and Tyrode solution, but is slightly decreased when the concentration of the serum reaches 25 per cent.
2. Fibroblasts cultivated in serum or in Tyrode solution are only in a condition of survival; they do not build up new protoplasm from the serum proteins and their mass does not increase.
3. When embryonic tissue juice is added to the medium, the tissues increase in mass. But the rate of growth is the same in media containing 0 per cent and 10 per cent serum. In 25 per cent serum, however, the rate of growth slightly decreases. Even in the presence of embryonic tissue juice, serum does not increase the rate of growth of connective tissue.
4. The nitrogenous compounds contained in serum are not used as food material by fibroblasts growing *in vitro*.

⁵ Lewis, M. R., and Lewis, W. H., *Anat. Rec.*, 1911, v, 277. Ingebrigtsen, R., *J. Exp. Med.*, 1912, xvi, 421. Burrows, M. T., *Anat. Rec.*, 1916-17, xi, 335. Burrows, M. T., and Neymann, C. A., *J. Exp. Med.*, 1917, xxv, 93.