HEAT AND GROWTH-INHIBITING ACTION OF SERUM.

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INTRODUCTION.

It is known that plasma or serum obtained from an adult animal restrains the growth of a pure culture of homologous fibroblasts.¹ This inhibiting power of plasma or serum is not apparent in very young animals, but manifests itself a few weeks after birth, increases progressively during adult life, and becomes very marked in old age. If it should restrain cell proliferation *in vivo* as much as *in vitro*, its rôle in many physiological and morbid processes would be important. Therefore, an investigation of its nature was begun. The purpose of the experiments described in this article was to study the modifications occurring in the rate of growth of fibroblasts when the serum composing the culture medium had been heated at various temperatures.

EXPERIMENTAL.

The serum was obtained from the plasma of chickens about 1 year old which had fasted for 24 hours. Its inhibiting action was determined by comparing the rate of growth of fibroblasts in a medium containing 50 per cent serum and in another containing 50 per cent Tyrode solution. The growth index of this serum averaged 0.9, which indicated that the inhibiting power was slight. The serum was used in the preparation of the medium in two different concentrations. When the fibrin of the coagulum was obtained from plasma, the medium was composed of 33.3 per cent plasma, 4 per cent tissue juice, and 62.6 per cent serum. When fibrinogen was used instead of plasma, the medium was made of 20 per cent fibrinogen suspension,²

¹ Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1921, **xxxiv**, 599.

² Ebeling, A. H., *J. Exp. Med.*, 1921, **xxxiii**, 641.

50 per cent serum, 27.5 per cent Tyrode solution, and 2.5 per cent tissue juice.

The action of unheated serum was compared with that of heated serum. Serum heated at 56°C. for a period of time varying from $\frac{1}{2}$ to 24 hours became slightly opalescent. When it was heated at 70°C. for $\frac{1}{2}$ hour, a precipitate occurred which was thrown down by centrifugation. The supernatant fluid was transparent, slightly opalescent, and not very viscous. The serum heated at 100°C. coagulated. The fluid which remained around the coagulum was used in the preparation of the medium.

The fibroblasts were taken from stock cultures of a 9 year old strain of connective tissue, and the cultures prepared in the usual manner. They were allowed to grow for 48 hours and the measurements were taken by outlining the original fragment and the new tissue under the projection apparatus and determining the area with the planimeter. The relative increase was calculated.³

1. *Action of Serum Heated at 56°C.*—In the first series of experiments, a comparison of the action of sera unheated and heated at 56°C. for 1 hour was made in media containing 33.3 per cent chicken plasma (Tables I and II). The rate of growth of fibroblasts in both media was identical.

In a second series of experiments, 20 per cent fibrinogen suspension was used instead of plasma. Differences in the rate of growth under the influence of heated and unheated sera became apparent (Tables III to VIII). The time during which the serum was kept at the temperature of 56°C. had little influence on its inhibiting action. The rate of growth of fibroblasts in sera heated at 56°C. for $\frac{1}{2}$ to 24 hours was decreased about 15 per cent.

2. *Action of Serum Heated at 70°C.*—In the first series of experiments, the action of serum heated at 70°C. for $\frac{1}{2}$ hour was compared with that of serum heated at 56°C. (Table IX). The rate of growth in the serum heated at 70°C. was 22 per cent slower than in the serum heated at 56°C.

In a second series of experiments, the action of sera unheated and heated at 70°C. was compared (Table X). The growth was found to

³ Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 231.

TABLE I.

Rate of Growth of Fibroblasts in Plasma and Serum, Unheated and Heated at 56°C.

Experiment No.	Culture No.	Width of ring.	
		Serum 62.6 per cent.	
		Unheated.	Heated.
1	23191	5.2	4.9
2	23192	4.9	5.1
3	23193	4.6	4.7
Average		4.9	4.9
4	1185-1, 1185-2	5.0	4.5
5	1185-3, 1185-4	4.0	4.0
Average		4.5	4.25
6	23194	5.0	4.5
7	23195	4.0	4.5
8	23196	5.0	5.0
Average		4.7	4.7

The serum used in the first three experiments was heated at 56°C. for 1 hour, in the remaining five experiments, for 2 hours. The rate of growth is expressed by the width of the ring of new tissue.

TABLE II.

Rate of Growth of Fibroblasts in Plasma and Serum, Unheated and Heated at 56°C.

Experiment No.	Culture No.	Relative increase.		Ratio: $\frac{\text{Heated serum}}{\text{Unheated serum}}$
		Serum 62.5 per cent.		
		Unheated.	Heated.	
1	23191	4.08	4.02	0.99
2	23192	3.76	3.91	1.04
3	23193	3.46	4.05	1.17
Average				1.07
4	23194	3.85	3.61	0.94
5	23196	3.82	4.07	1.07
Average				1.00

In the first group of experiments, the serum was heated at 56°C. for 1 hour, in the second group, for 2 hours. The rate of growth is expressed by the relative increase of the tissue.

TABLE III.

Rate of Growth of Fibroblasts in Serum, Unheated and Heated at 56°C. for $\frac{1}{2}$ Hr.

Experiment No.	Culture No.	Relative increase.		Ratio: $\frac{\text{Heated serum}}{\text{Unheated serum}}$
		Serum 50 per cent.		
		Unheated.	Heated.	
1	20936	3.36	2.72	0.80
2	20937	2.13	1.62	0.76
3	20938	3.50	2.68	0.77
4	21491	5.06	4.87	0.96
5	21492	3.43	2.93	0.85
6	21493	4.80	3.43	0.71
7	21450	2.76	2.51	0.91
8	21841	2.90	2.20	0.76
9	21842	2.60	2.40	0.92
10	21843	2.80	2.30	0.82
11	21844	2.40	2.40	1.00
12	23110	2.16	1.89	0.88
Average		3.16	2.66	0.85

TABLE IV.

Rate of Growth of Fibroblasts in Serum, Unheated and Heated at 56°C. for 1 Hr.

Experiment No.	Culture No.	Relative increase.		Ratio: $\frac{\text{Heated serum}}{\text{Unheated serum}}$
		Serum 50 per cent.		
		Unheated.	Heated.	
1	23164	3.72	3.42	0.92
2	23165	3.73	3.37	0.90
3	23166	2.91	2.91	1.00
4	23167	2.48	2.30	0.93
5	23226	3.55	4.00	1.12
6	23227	4.35	4.47	1.03
7	23246	2.28	1.81	0.79
8	23248	2.74	2.00	0.73
9	23249	2.21	1.85	0.84
Average				0.92
10	23477	5.12	4.64	0.91
11	23478	3.92	3.55	0.91
12	23479	3.80	3.12	0.82
13	23944	2.81	2.22	0.79
14	23945	3.20	2.65	0.83
15	23946	3.14	2.56	0.82
Average				0.85

In the first nine experiments, 5 per cent embryo juice was used, and in the remaining six experiments, 2.5 per cent embryo juice.

TABLE V.

Rate of Growth of Fibroblasts in Serum, Unheated and Heated at 56°C. for 2 Hrs.

Experiment No.	Culture No.	Relative increase.		Ratio: $\frac{\text{Heated serum}}{\text{Unheated serum}}$
		Serum 50 per cent.		
		Unheated.	Heated.	
1	23168	3.12	2.77	0.89
2	23169	3.00	2.74	0.91
3	23170	3.19	2.67	0.84
4	23171	3.10	2.83	0.91
5	23228	3.50	3.22	0.92
6	23229	3.93	3.80	0.97
7	23251	3.24	2.85	0.88
8	23252	2.55	2.33	0.91
9	23253	3.32	3.00	0.90
Average				0.90

In the first two experiments, 5 per cent embryo juice was used, and in the remaining seven experiments, 2.5 per cent embryo juice.

TABLE VI.

Rate of Growth of Fibroblasts in Serum, Unheated and Heated at 56°C. for 6 Hrs.

Experiment No.	Culture No.	Relative increase.		Ratio: $\frac{\text{Heated serum}}{\text{Unheated serum}}$
		Serum 50 per cent.		
		Unheated.	Heated.	
1	23288	3.21	2.70	0.84
2	23289	4.21	3.20	0.76
3	23290	4.05	3.21	0.79
Average				0.80

TABLE VII.

Rate of Growth of Fibroblasts in Serum, Unheated and Heated at 56°C. for 12 Hrs.

Experiment No.	Culture No.	Relative increase.		Ratio: $\frac{\text{Heated serum}}{\text{Unheated serum}}$
		Serum 50 per cent.		
		Unheated.	Heated.	
1	23291	3.50	2.78	0.79
2	23292	3.70	3.11	0.84
3	23293	3.67	3.22	0.88
Average				0.84

TABLE VIII.

Rate of Growth of Fibroblasts in Serum, Unheated and Heated at 56°C. for 24 Hrs.

Experiment No.	Culture No.	Relative increase.		Ratio: $\frac{\text{Heated serum}}{\text{Unheated serum}}$
		Serum 50 per cent.		
		Unheated.	Heated.	
1	23440	3.70	3.08	0.83
2	23441	3.58	2.93	0.82
3	23442	3.87	3.03	0.78
4	23334	3.07	2.90	0.94
5	23335	3.26	3.15	0.96
6	23336	3.48	2.90	0.83
Average				0.86

TABLE IX.

Rate of Growth of Fibroblasts in Serum Heated at 56°C. for $\frac{1}{2}$ Hr. and at 70°C. for $\frac{1}{2}$ Hr.

Experiment No.	Culture No.	Relative increase.		Ratio: $\frac{\text{Serum heated at } 70^{\circ}\text{C.}}{\text{Serum heated at } 56^{\circ}\text{C.}}$
		Serum 50 per cent.		
		Heated at 56°C.	Heated at 70°C.	
1	23812	2.70	1.98	0.73
2	23813	2.33	2.00	0.86
3	23814	3.06	2.56	0.84
4	23846	2.70	2.18	0.81
5	23847	2.56	1.92	0.75
6	23848	2.76	2.08	0.75
7	23886	3.26	2.76	0.85
8	23887	2.67	2.37	0.89
9	23888	3.00	2.20	0.73
10	23941	3.40	2.44	0.72
11	23942	3.40	2.43	0.71
12	23943	3.78	2.80	0.74
13	23947	2.39	1.74	0.73
14	23948	2.33	1.81	0.78
15	23949	2.84	2.28	0.80
Average				0.78

TABLE X.

Rate of Growth of Fibroblasts in Serum, Unheated and Heated at 70°C. for $\frac{1}{4}$ Hr.

Experiment No.	Culture No.	Relative increase.		Ratio: $\frac{\text{Heated serum}}{\text{Unheated serum}}$
		Serum 50 per cent.		
		Unheated.	Heated.	
1	23337	3.20	2.28	0.71
2	23338	4.33	3.14	0.73
3	23339	4.24	2.69	0.63
4	23443	3.91	2.08	0.53
5	23444	3.43	1.69	0.49
6	23445	3.95	1.69	0.43
7	23709	3.62	2.25	0.62
8	23710	3.90	2.57	0.66
9	23711	3.90	2.60	0.67
10	23679	2.38	1.94	0.82
11	23680	2.21	1.80	0.81
12	23681	3.88	2.93	0.76
Average				0.66

be 34 per cent slower in heated than in unheated serum. Both series of experiments showed almost identical results. It must be observed also (Tables IX and X) that in both series the values for the relative increase of the tissues in serum heated at 70°C. are about similar. The consistency of the results demonstrates that there were no technical errors.

TABLE XI.

Rate of Growth of Fibroblasts in Serum Heated at 100°C. for 10 Min. and at 70°C. for $\frac{1}{4}$ Hr.

Experiment No.	Culture No.	Relative increase.		Ratio: $\frac{\text{Heated at } 70^{\circ}\text{C.}}{\text{Heated at } 100^{\circ}\text{C.}}$
		Serum 50 per cent.		
		Heated at 100°C.	Heated at 70°C.	
1	23889	3.35	2.30	0.69
2	23890	2.60	1.90	0.73
3	23891	3.26	2.55	0.78
Average				0.73

TABLE XII.
Rate of Growth of Fibroblasts in Serum, Unheated and Heated at 100°C. for 10 Min.

Experiment No.	Culture No.	Relative increase.		Ratio: $\frac{\text{Heated serum}}{\text{Unheated serum}}$
		Serum 50 per cent.		
		Unheated.	Heated.	
1	23632	3.12	3.06	0.98
2	23633	3.43	3.01	0.88
3	23634	3.19	3.02	0.95
4	23635	2.88	2.85	0.99
5	23654	2.91	2.87	0.99
6	23655	3.05	3.44	1.13
7	23656	3.32	3.14	0.94
8	23657	3.30	2.90	0.88
9	23849	4.03	3.25	0.81
10	23850	3.16	2.74	0.87
11	23851	3.81	3.56	0.93
Average				0.94

TEXT-FIG. 1. Variations in the rate of growth of fibroblasts in function of the temperature at which the serum was heated. The rate of growth of fibroblasts in unheated serum was considered to be equal to 100 per cent.

3. *Action of Serum Heated at 100°C.*—The fluid remaining after coagulation was compared with serum heated at 70°C. and with unheated serum. The figures show that the rate of growth in serum heated at 100°C. was 27 per cent faster than in serum heated at 70°C.; that is, about equal to the growth rate in unheated serum (Table XI). The experiments in which a comparison was made between the respective actions of unheated serum and serum heated at 100°C. confirmed these results (Table XII). The amount of growth was practically identical in both unheated serum and serum heated at 100°C. It must be remembered that differences equal to or smaller than 10 per cent may be due to experimental errors.

A curve was plotted expressing the variations of the rate of growth of the fibroblasts in function of the temperature at which the serum had been heated (Text-fig. 1). It shows that the rate of cell proliferation decreased after the serum had been heated at 56°C., and became still slower after it had been heated at 70°C. A marked increase in the rate of growth took place after the serum had been heated at 100°C.

DISCUSSION.

The results obtained in the preceding experiments may be summarized as follows:

The action of heat at 56°C. increased by 15 per cent the inhibiting action of serum obtained from young adult chickens on the proliferation of fibroblasts. The action of heat at 70°C. increased the inhibiting action by 34 per cent. When the serum had been heated at 100°C., its inhibiting action became about equal to that of non-heated serum. Therefore, heated serum contained a factor which markedly inhibited the growth of fibroblasts and which developed at, or resisted a temperature of 70°C.

These experiments confirmed the results obtained by Ingebrigtsen⁴ in his study of the growth of guinea pig bone marrow in homologous serum unheated and heated at 56°C. The growth of bone marrow was found to be more extensive in unheated than in heated serum. The differences in the action of both sera were more striking than in

⁴ Ingebrigtsen, R., *J. Exp. Med.*, 1912, xv, 397.

our experiments. This was due to the fact that Ingebrigtsen used a medium containing a very large amount of serum and observed lymphocytes instead of fibroblasts.

The increase of the inhibiting power of serum after it had been heated at 56° and 70°C. may be considered as due to the production, under the influence of heat, of a change which renders the serum more toxic for the homologous fibroblasts. But it may also be attributed to the destruction of substances presenting the same heat resistance as complement and amboceptor, and partly protecting the cells against the inhibiting action of a third substance resisting heat at 70°C. Serum modified by heat acts in an opposite manner on heterologous tissues. Heated serum is a better culture medium for heterologous cells than unheated serum, as has already been shown by Ingebrigtsen.⁴ We have found lately that the inhibiting action of dog, rabbit, and cat serum, heated at 56° and 66°C., on the rate of multiplication of fibroblasts is very much decreased. It seems that the factors which protect the organism against foreign cells and bacteria might also oppose the growth-inhibiting factor of serum and allow the cells to display a greater activity.

CONCLUSIONS.

The inhibiting action of homologous serum on the proliferation of fibroblasts *in vitro* was increased after the serum had been heated at 56° and 70°C. This action decreased after the serum had been heated at 100°C.