AGE AND MULTIPLICATION OF FIBROBLASTS.

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The symptoms of senescence are evidently the expression of profound physicochemical changes which occur in the humors and the tissues of the organism under the influence of time. But the nature of these changes is still utterly unknown. If the modifications of blood serum and interstitial lymph which are functions of the age of the organism could be quantitatively studied, it is probable that the mechanism of growth, differentiation, and senescence would be better understood. Some years ago, one of the writers attempted to develop a method for detecting these modifications. By cultivating connective tissue in the plasma of chickens of different ages he found that the growth was more abundant in the plasma of the younger than in that of the older animals.[1] This fact suggested that proliferating fibroblasts could be used as a reagent for the changes occurring in the blood under the influence of age, if a technique sufficiently accurate were developed. During the last few years, the necessary improvements in the methods of cultivating tissues have been realized.[2,3] It has, then, become possible to study the cause of the slower multiplication of fibroblasts in the serum of old animals and the factors of senescence. The experiments of Loeb and Northrop on the temperature coefficient of duration of life of *Drosophila* lead to the conclusion that the duration of life was probably determined by the production of a substance leading to old age, or by the destruction of a substance which normally prevents old age and natural death.[4] It was important to know which of these

[1] Carrel, A., *J. Exp. Med.*, 1913, xviii, 287.

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

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

[4] Loeb, J., and Northrop, J. H., *J. Biol. Chem.*, 1917, xxxii, 103.

hypotheses agrees with the reality. The purpose of the experiments described in this paper was to ascertain whether a definite relation exists between the rate of multiplication of fibroblasts cultivated in plasma and the age of the animal from which the plasma is obtained, and whether the modifications brought about by age in the action of the plasma on fibroblasts are due to the disappearance of an accelerating factor, or to the production of an inhibiting factor.

I.

Relation of Age of Serum to Rate of Growth and Duration of Life of Fibroblasts.

While it has been known for several years that the rate of growth of connective tissue varies in inverse ratio to the age of the animal from

TABLE I.

Rate of Growth of Embryonic Heart and Liver in the Plasma of Chickens 2 Years, 5 Years, and 4 Months Old.

Experiment No.	Culture No.	Date.	Nature of tissue.	Width of ring of new tissue.			Remarks.
				2 yr. old chicken plasma.	5 yr. old chicken plasma.	4 mo. old chicken plasma.	
		1912					
1	2321–1	Sept. 17	8 day embryo heart.	2.0		4.0	The plasma
2	2321–2	" 17	8 " " "	1.5–2.0		3.5–4.0	was taken
3	2321–3	" 17	8 " " "	1.5–2.0		3.5–4.0	from the animals, Sept. 17, 1912.
4	2326	" 18	7 " " "		1.5	3.0	
5	2335–1	" 20	7 " " "		2.0	3.0	
6	2335–2	" 20	7 " " liver.		1.2	2.5–3.0	
7	2335–3	" 20	7 " " "		1.0	2.5	
8	2342	" 21	7 " " heart.		2.0	3.0	
9	2356	" 23	7 " " "		2.0	2.5	

which the plasma is taken,[1] the relation between the two quantities could not be established, owing to the lack of sufficient accuracy of the technique. In 1912, fragments of embryonic heart and liver were cultivated in plasma obtained from 4 month, 2 year, and 5 year old chickens (Table I). The width of the ring of connective tissue pro-

duced around the fragments was larger in the plasma of the young than in that of the older chickens. A larger amount of connective tissue was produced in the serum of a 1 month old kitten than in that of a 9 year old cat. The same phenomenon occurred when the sera of 20 and 45 year old human beings were used as media. Con-

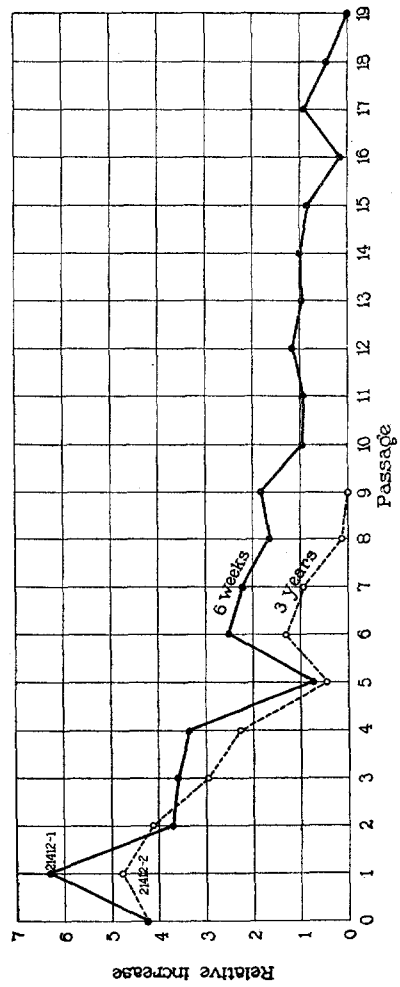
TABLE II.

Rate of Growth and Duration of Life of Fibroblasts in the Plasma of a 6 Week Old Chicken and a 3 Year Old Hen.

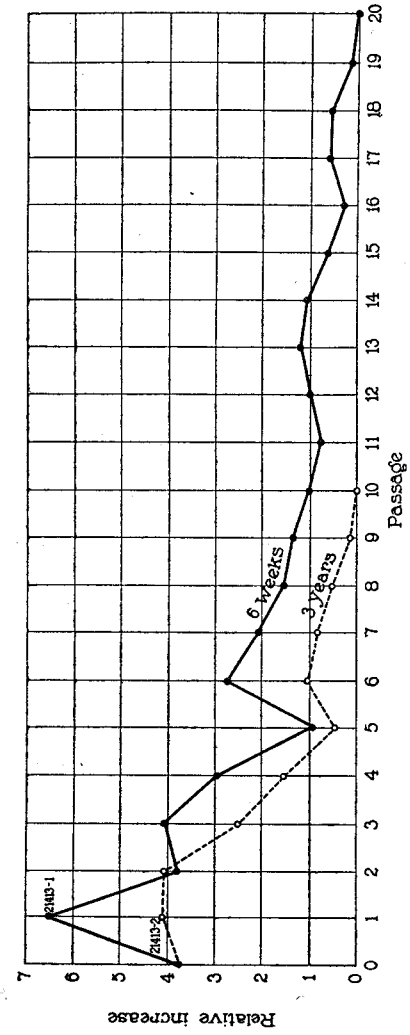
Experiment 1.					Experiment 2.					Experiment 3.				
Passage No.	Culture No.	Date.	Relative increase. Young plasma.	Relative increase. Adult plasma.	Passage No.	Culture No.	Date.	Relative increase. Young plasma.	Relative increase. Adult plasma.	Passage No.	Culture No.	Date.	Relative increase. Young plasma.	Relative increase. Adult plasma.
		1921					*1921*					*1921*		
1	21412	May 12	6.3	4.78	1	21413	May 12	6.54	4.12	1	21414	May 12	6.06	3.86
2	21444	" 14	3.7	4.13	2	21446	" 14	3.8	4.09	2	21448	" 14	3.59	3.74
3	21485	" 16	3.61	2.97	3	21487	" 16	4.08	2.5	3	21489	" 16	4.47	2.85
4	21522	" 18	3.36	2.26	4	21524	" 18	2.96	1.55	4	21526	" 18	4.5	1.26
5	21548	" 20*	0.75	0.45	5	21550	" 20*	0.95	0.47	5	21552	" 20*	1.22	0.45
6	21582	" 21	2.52	1.31	6	21584	" 21	2.75	1.04	6	21586	" 21	2.37	1.22
7	21598	" 23	2.22	0.91	7	21600	" 23	2.08	0.85	7	21602	" 23	2.09	0.62
8	21636	" 25	1.64	0.13	8	21638	" 25	1.56	0.53	8	21640	" 25	1.96	0.42
9	21672	" 27	1.82	0	9	21674	" 27	1.35	0.21	9	21676	" 27	1.26	0.33
10	21714	" 29	0.97		10	21716	" 29	1.62	0	10	21718	" 29	1.09	0
11	21732	" 31	0.93		11	21733	" 31	0.76		11	21734	" 31	0.98	
12	21765	June 2	1.17		12	21766	June 2	1.0		12	21767	June 2	1.02	
13	21795	" 4	0.97		13	21796	" 4	1.21		13	21797	" 4	0.71	
14	21823	" 6	1.0		14	21824	" 6	1.08		14	21825	" 6	0.85	
15	21857	" 8	0.85		15	21858	" 8	0.61		15	21859	" 8	1.0	
16	21876	" 10	0.15		16	21877	" 10	0.27		16	21878	" 10	0.3	
17	21894	" 11	0.9		17	21895	" 11	0.60		17	21896	" 11	0.4	
18	21906	" 13	0.45		18	21907	" 13	0.36		18	21908	" 13	0.48	
19	21925	" 15	0		19	21926	" 15	0.15		19	21927	" 15	0.5	
					20	21947	" 17	0		20	21948	" 17†	0.74	
										21	21974	" 20	0.25	
										22	21992	" 22	0.26	

* 24 hour culture.

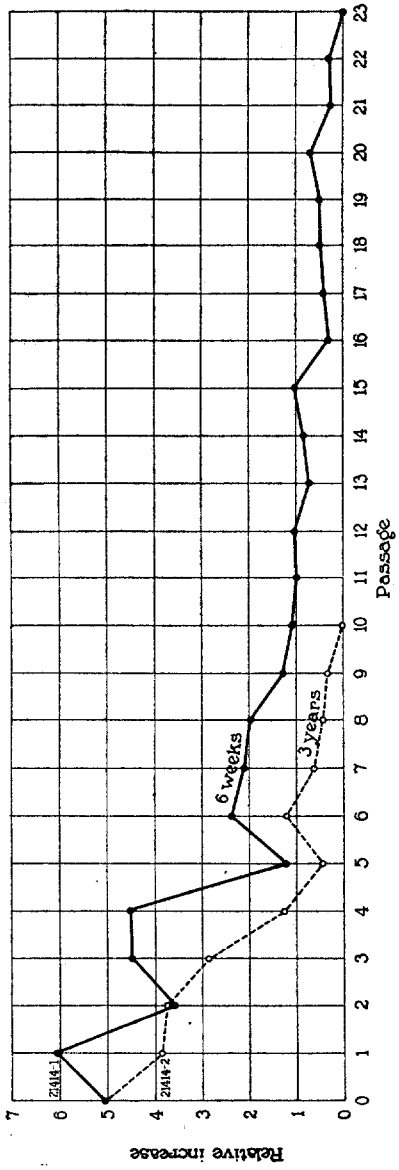
† 72 hour culture.



TEXT-FIG. 1. Experiment 1. Comparison of the rate of growth of fibroblasts in the plasma of a 6 week old chicken and a 3 year old hen. The sudden drop in the rate of growth of the culture in the 6 week plasma was accidental. The small amount of growth obtained at the fifth passage was due to the transfer of the tissues to a fresh medium after 24 hours instead of 48 hours.



TEXT-FIG. 2. Experiment 2. Comparison of the rate of growth of fibroblasts in the plasma of a 6 week old chicken and a 3 year old hen. The sudden drop in the rate of growth of the culture in the 6 week plasma was accidental. The small amount of growth obtained at the fifth passage was due to the transfer of the tissues to a fresh medium after 24 hours instead of 48 hours.



TEXT-FIG. 3. Experiment 3. Comparison of the rate of growth of fibroblasts in the plasma of a 6 week old chicken and a 3 year old hen. The sudden drop in the rate of growth of the culture in the 6 week plasma was accidental. The small amount of growth obtained at the fifth passage was due to the transfer of the tissues to a fresh medium after 24 hours instead of 48 hours.

nective tissue always grew more abundantly in the serum of the younger animals. In spite of the defects of the technique, the influence of age was evident.

Another series of experiments was made recently with more precise measurements. Pure cultures of fibroblasts were taken from a 9 year old strain of connective tissue previously kept in a mixture of plasma and embryo juice. Each fragment was divided in two equal parts which were imbedded in the plasma of chickens 6 weeks to 9 years of age. The proliferation of the fibroblasts in young and older plasmas was studied according to a technique previously described.[3] 1 hour after the preparation of the cultures, and 48 hours later, the fragments were outlined and their area was measured. The rate of growth was expressed in terms of the relative increase of the area of the original fragment in 48 hours. A study was made of the rate of growth and the duration of life of the fibroblasts in the plasma of 6 week, 3 month, 3 year, and 9 year old chickens.

1. Plasma of 6 Week Old and 3 Year Old Chickens.—The plasma of animals 6 weeks and 3 years of age was used as a medium for fibroblasts taken from a 24 hour old stock culture. In every experiment, the growth was more active in the young than in the older plasma (Table II). On account of the accidental irregularities of the curves, the comparison of the rates of growth was made after the fifth passage (Text-figs. 1 to 3). The average amount of tissue produced during this period in the plasma of the older animal was 35 per cent of that in the plasma of the young animal. It was also found that the duration of life of the fibroblasts in the plasma of the older animal was 46 per cent of that in the plasma of the younger animal.

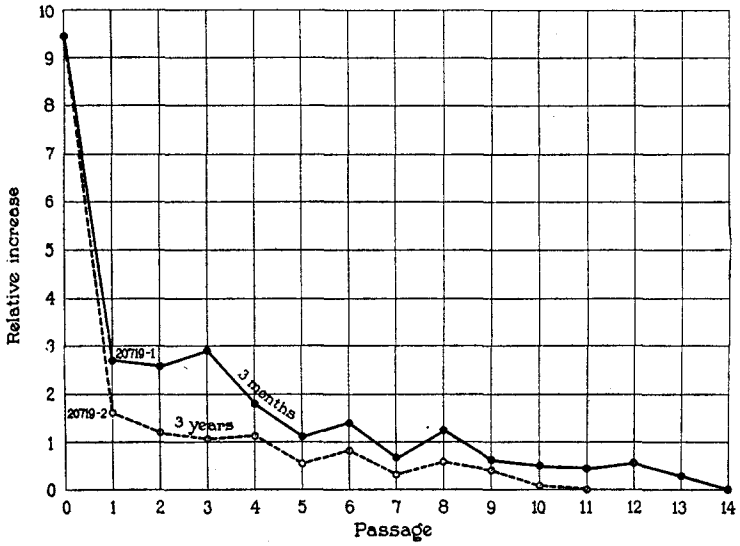
2. Plasma of 3 Month Old and 3 Year Old Chickens.—A comparison of the plasmas of 3 month and 3 year old animals was made in the same manner (Table III). The fibroblasts were taken from a 48 hour old stock culture. As soon as they were transferred from the mixture of plasma and embryo juice to plasma alone, a sudden drop in the velocity of growth was observed, as is shown by the descending branch of the curves (Text-figs. 4 to 7). This resulted from the suppression in the medium of the active substances contained in the

TABLE III.

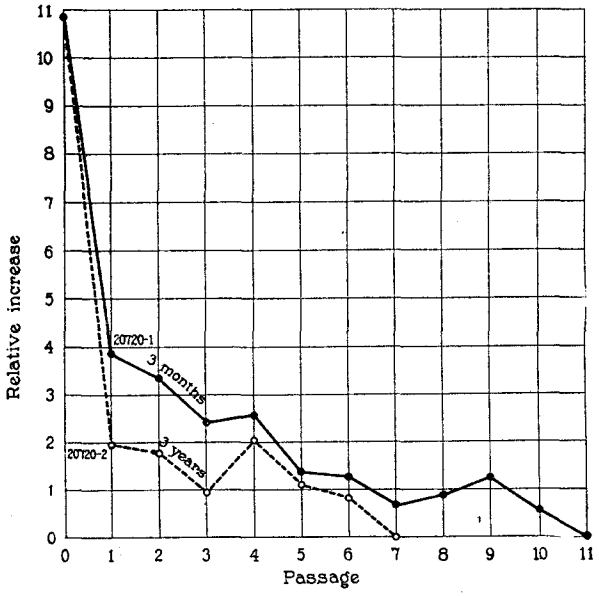
Rate of Growth and Duration of Life of Fibroblasts in the Plasma of a 3 Month Old Chicken and a 3 Year Old Hen.

Experiment 1.					Experiment 2.					Experiment 3.					Experiment 4.				
			Relative increase.					Relative increase.					Relative increase.					Relative increase.	
Passage No.	Culture No.	Date.	Young plasma.	Adult plasma.	Passage No.	Culture No.	Date.	Young plasma.	Adult plasma.	Passage No.	Culture No.	Date.	Young plasma.	Adult plasma.	Passage No.	Culture No.	Date.	Young plasma.	Adult plasma.
		1921					*1921*					*1921*					*1921*		
1	20719	Apr. 12	2.7	1.64	1	20720	Apr. 12	3.87	1.98	1	20721	Apr. 12	3.43	1.48	1	20722	Apr. 12	4.28	1.49
2	20779	" 14	2.6	1.22	2	20781	" 14	3.36	1.78	2	20783	" 14	2.73	1.81	2	20785	" 14	3.14	1.8
3	20846	" 16	2.9	1.06	3	20848	" 16	2.42	0.98	3	20850	" 16	2.98	1.75	3	20852	" 16	2.73	1.22
4	20880	" 18	1.8	1.12	4	20882	" 18	2.57	2.03	4	20884	" 18	2.13	1.17	4	20886	" 18	2.23	1.43
5	20928	" 20	1.1	0.53	5	20930	" 20	1.39	1.11	5	20932	" 20	1.82	1.21	5	20934	" 20	1.76	0.94
6	20993	" 22	1.4	0.83	6	20995	" 22	1.27	0.81	6	20997	" 22	1.63	0.78	6	20999	" 22	1.74	0.43
7	21067	" 25*	0.66	0.31	7	21069	" 25*	0.69	0	7	21071	" 25*	1.11	0	7	21073	" 25*	1.16	0
8	21113	" 27	1.24	0.61	8	21115	" 27	0.88		8	21116	" 27	0.97		8	21117	" 27	1.44	
9	21156	" 29	0.65	0.4	9	21158	" 29	1.24		9	21159	" 29	1.08		9	21160	" 29	1.43	
10	21121	May 2*	0.5	0.09	10	21123	May 2*	0.58		10	21224	May 2*	0.39		10	21225	May 2*	0.28	
11	21259	" 4	0.44	0	11	21261	" 4	0		11	21262	" 4	0.42		11	21263	" 4	0.68	
12	21298	" 6	0.56							12	21299	" 6	0.3		12	21300	" 6	0.27	
13	21338	" 8	0.29							13	21339	" 8	0.2		13	21340	" 8	0.6	
14	21375	" 10	0							14	21376	" 10	0		14	21377	" 10	0.28	
															15	21408	" 12	0	

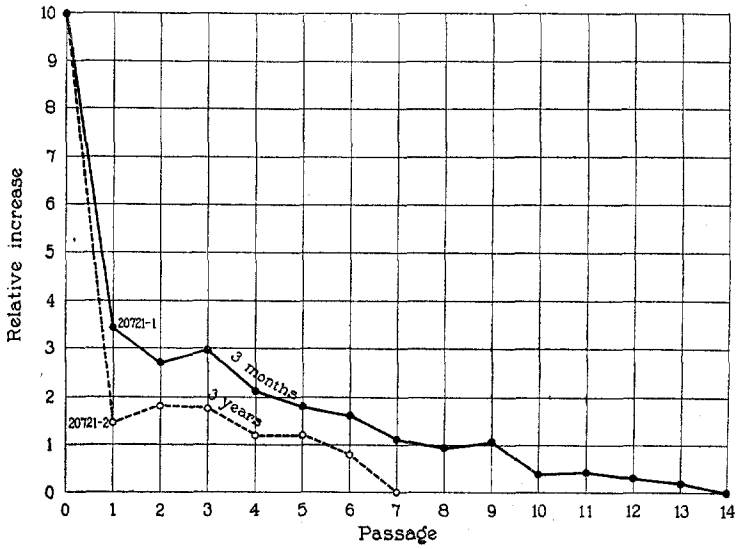
* 72 hour culture.



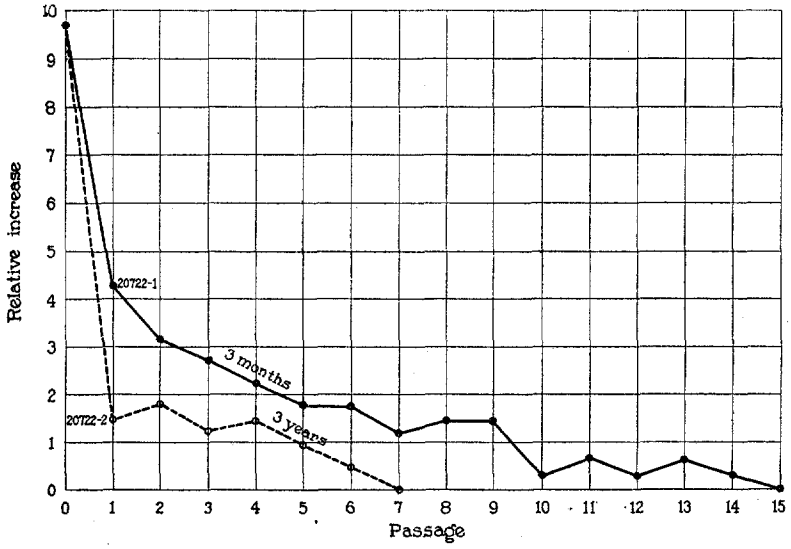
TEXT-FIG. 4. Experiment 1. Comparison of the rate of growth of fibroblasts in the plasma of a 3 month old chicken and a 3 year old hen.



TEXT-FIG. 5. Experiment 2. Comparison of the rate of growth of fibroblasts in the plasma of a 3 month old chicken and a 3 year old hen.



TEXT-FIG. 6. Experiment 3. Comparison of the rate of growth of fibroblasts in the plasma of a 3 month old chicken and a 3 year old hen.



TEXT-FIG. 7. Experiment 4. Comparison of the rate of growth of fibroblasts in the plasma of a 3 month old chicken and a 3 year old hen.

embryo juice.[5,6] After the first passage of both fragments in the plasma of the young and older chickens, a marked and almost con-

TABLE IV.

Rate of Growth and Duration of Life of Fibroblasts in the Plasma of a 3 Month Old Chicken and a 9 Year Old Cock.

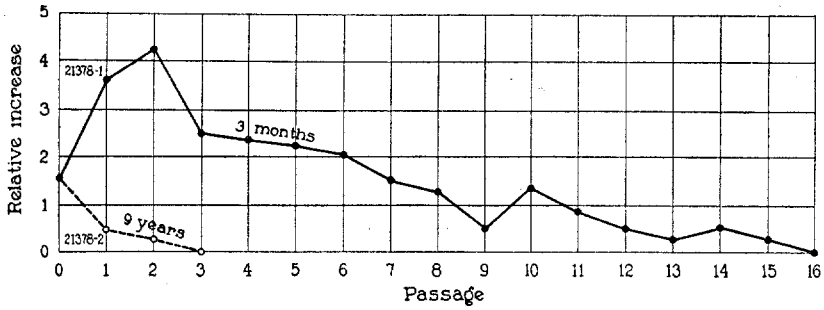
Experiment 1.					Experiment 2.					Experiment 3.				
			Relative increase.					Relative increase.					Relative increase.	
Passage No.	Culture No.	Date.	Young plasma.	Adult plasma.	Passage No.	Culture No.	Date.	Young plasma.	Adult plasma.	Passage No.	Culture No.	Date.	Young plasma.	Adult plasma.
		1921					1921					1921		
1	21244	May 3	3.65	0.48	1	21245	May 3	2.14	0.55	1	21246	May 3	4.56	2.08
2	21279	" 5	4.28	0.28	2	21281	" 5	3.62	0	2	21283	" 5	4.62	1.04
3	21318	" 7	2.52	0	3	21320	" 7	2.46		3	21321	" 7	3.17	0
4	21378	" 10	2.38		4	21379	" 10	1.84		4	21380	" 10	2.07	
5	21409	" 12	2.27		5	21410	" 12	2.15		5	21411	" 12	2.0	
6	21441	" 14	2.09		6	21442	" 14	1.58		6	21443	" 14	1.48	
7	21482	" 16	1.53		7	21483	" 16	1.50		7	21484	" 16	1.29	
8	21519	" 18	1.29		8	21520	" 18	1.56		8	21521	" 18	1.38	
9	21545	" 20*	0.55		9	21546	" 20*	0.61		9	21547	" 20*	0.49	
10	21579	" 21	1.34		10	21580	" 21	1.63		10	21581	" 21	2.63	
11	21595	" 23	0.83		11	21596	" 23	0.75		11	21597	" 23	0.49	
12	21633	" 25	0.50		12	21634	" 25	0.61		12	21635	" 25	0.25	
13	21669	" 27	0.26		13	21670	" 27	0.45		13	21671	" 27	0.17	
14	21711	" 29	0.52		14	21712	" 29	0.68		14	21713	" 29	0.3	
15	21729	" 31	0.26		15	21780	" 31	0.51		15	21731	" 31	0	
16	21763	June 2	0		16	21764	June 2	0						

* 24 hour culture.

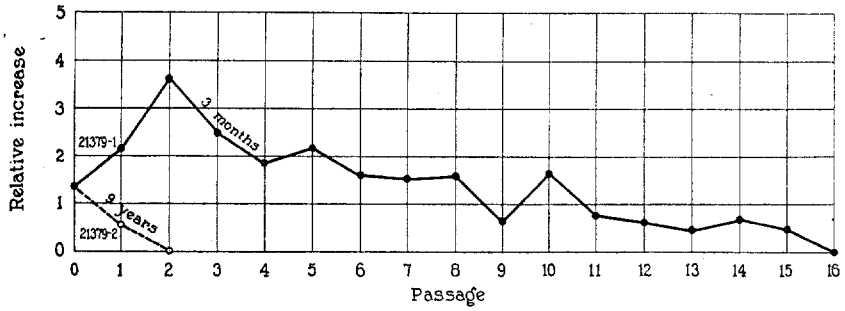
stant difference in the extent of growth was observed. The number of passages during which the fibroblasts multiplied, that is, the duration of the life of the cultures in both plasmas, differed widely. The tissues cultivated in the plasma of the older animal died after the seventh passage on an average, while in the plasma of the young

[5] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 317.

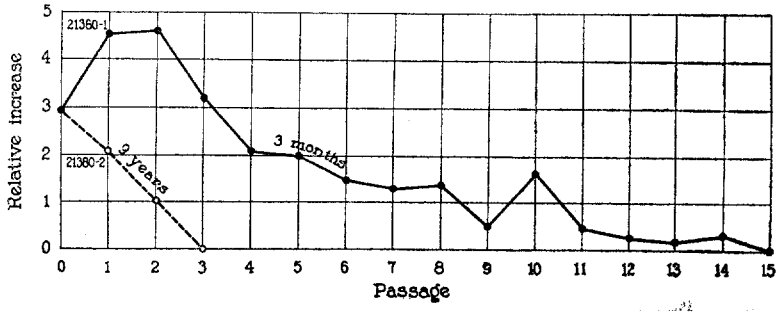
[6] Carrel, A., *J. Exp. Med.*, 1913, xvii, 14.



TEXT-FIG. 8. Experiment 1. Comparison of the rate of growth of fibroblasts in the plasma of a 3 month old chicken and a 9 year old cock.

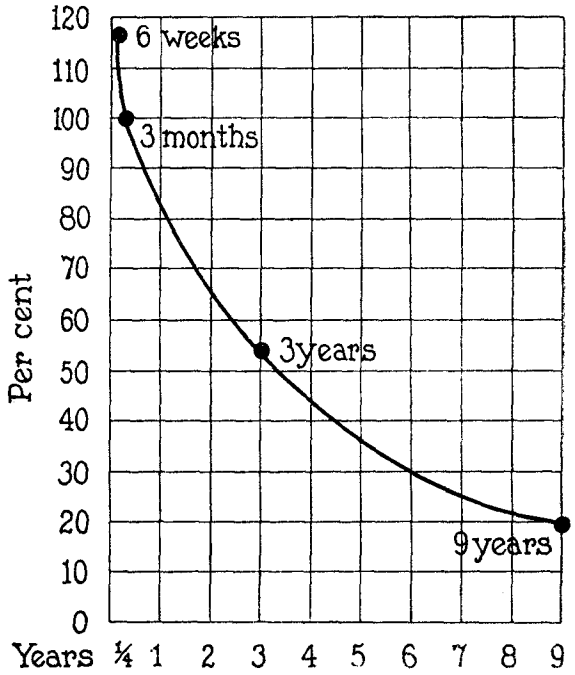


TEXT-FIG. 9. Experiment 2. Comparison of the rate of growth of fibroblasts in the plasma of a 3 month old chicken and a 9 year old cock.



TEXT-FIG. 10. Experiment 3. Comparison of the rate of growth of fibroblasts in the plasma of a 3 month old chicken and a 9 year old cock.

animal, death occurred after the thirteenth passage. Thus, the duration of life of connective tissue in the 3 year old plasma was 54 per cent of that in the 3 month old plasma (Text-fig. 11). The amount of tissue produced in a given time in both plasmas showed striking differences. A comparison was made between the areas of new



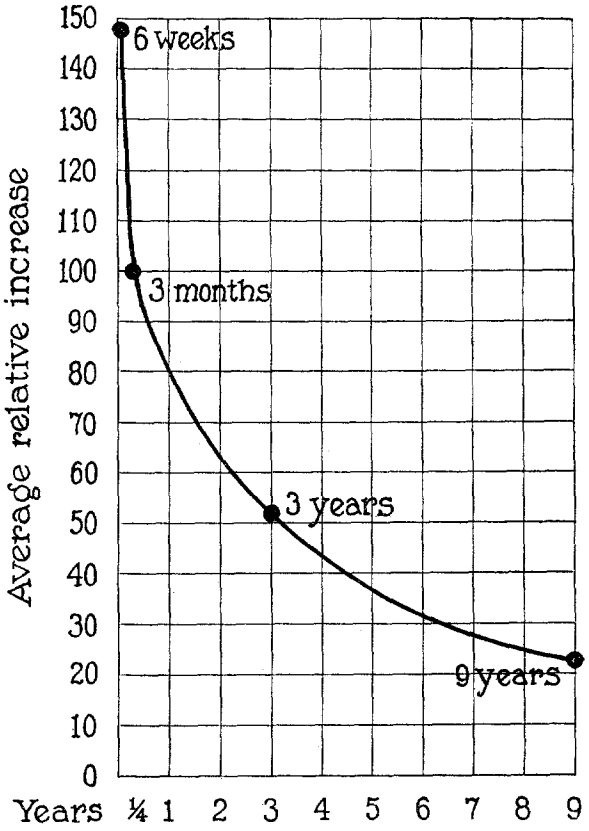
TEXT-FIG. 11. Duration of life *in vitro* of fibroblasts in function of the age of the animals from which the plasma was taken.

tissue produced in both plasmas during the life of the fibroblasts in the older plasma. The amount of growth in the plasma of the 3 year old animal was 52 per cent of that in the plasma of the 3 month old animal (Text-fig. 12).

3. Plasma of 3 Month Old and 9 Year Old Chickens.—The action of the plasma of a 3 month old chicken was compared with that of the plasma of a 9 year old chicken (Table IV, Text-figs. 8 to 10). The rate of growth in the old plasma was 23 per cent of that in the young

plasma (Text-fig. 12). The duration of life of the cultures in the plasma of the old animal was only 19 per cent of that in the plasma of the young animal (Text-fig. 11).

4. Serum of 3 Month Old and 3 Year Old Chickens.—An investiga-



TEXT-FIG. 12. Rate of growth of fibroblasts in function of the age of the animal from which the plasma was taken.

tion was then made in order to determine whether serum acted in the same manner as plasma. Fragments of a pure culture of fibroblasts were placed in media composed of fibrinogen 20 per cent, Tyrode solution 10 per cent, and serum 70 per cent (Table V). The sera were obtained from the plasmas of the 3 month and 3 year old

animals. It was soon found that the original fragment often contracted in the serum of the older animals, and no accurate measurement was possible. In several cases, however, the cultures remained normal. The rate of growth during the first passage in the serum of the 3 year old chicken was 69 per cent of that in the serum of the 3 month old chicken. Other experiments were made in which the fibrinogen suspension was replaced in the medium by 30 per cent normal plasma. Again similar changes in the rate of growth were observed.

TABLE V.

Rate of Growth of Fibroblasts in the Serum of a 3 Month Old and a 3 Year Old Hen.

Experiment No.	Culture No.	Date.	Medium.	
			Young.	Adult.
			Serum 70 per cent; fibrinogen 20 per cent.	Serum 70 per cent; fibrinogen 20 per cent.
		1921		
1	20839	Apr. 16	2.28	1.44
2	20840	" 16	2.65	1.74
3	20841	" 16	1.91	1.47
4	20842	" 16	3.06	2.14
5	20843	" 16	3.03	2.42
6	20844	" 16	2.34	1.55
7	20845	" 16	3.13	1.89
Average.....			2.63	1.81

There was, therefore, no doubt that the rate of multiplication of fibroblasts and the duration of their life *in vitro* varied in inverse ratio to the age of the animals from which the plasma or the serum was taken. The graph representing the rate of growth, in function of the age of the animals which have given the plasma, shows that the differences are very large (Text-fig. 12). Another graph expresses the relation between the duration of life and the age (Text-fig. 11). These curves show that there is a definite relation between the rate of proliferation of the fibroblasts, the length of their life *in vitro*, and the age of the animal. It is, therefore, possible to use a pure culture of

fibroblasts as a reagent for detecting some of the modifications occurring in blood serum under the influence of time.

II.

Relation of Concentration of Serum to Rate of Growth.

The action on the growth of fibroblasts of the serum taken from animals of advancing age could be explained by the disappearance from the blood of an accelerating factor, or the development in the blood of an inhibiting factor. If youth were supposed to be characterized by a factor present in the blood and activating the multiplication of fibroblasts, and senescence by a decrease in the power of this factor, connective tissue cells should proliferate more

TABLE VI.

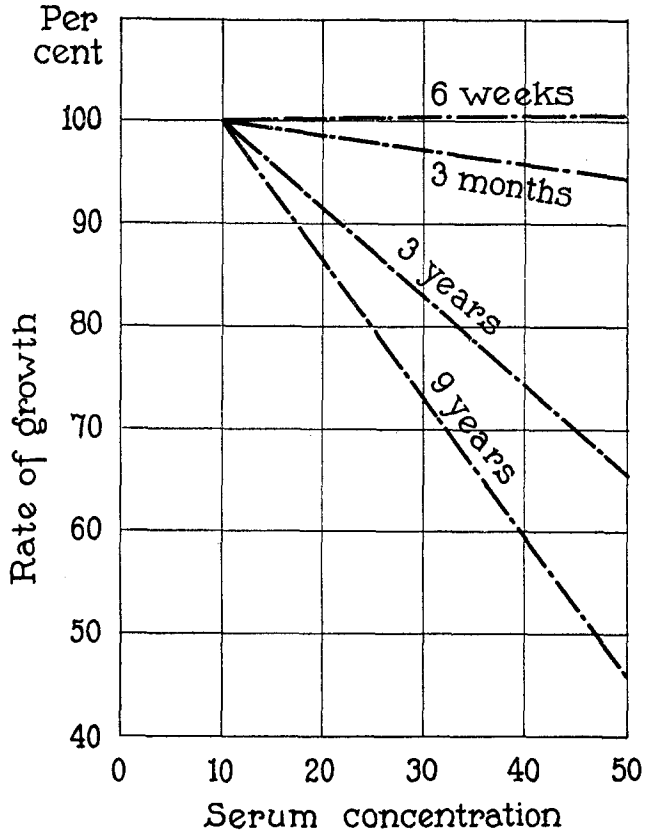
Rate of Growth of Fibroblasts in High and Low Concentrations of the Serum of a 6 Week Old Chicken.

Experiment No.	Culture No.	Date.	Medium.	
			Serum 10 per cent; fibrinogen 20 per cent.	Serum 50 per cent; fibrinogen 20 per cent.
		1921		
1	21424	May 13	4.34	3.90
2	21425	" 13	2.62	3.17
3	21426	" 13	3.74	4.21
4	21427	" 13	4.13	4.00
5	21428	" 13	3.58	3.35
Average			3.68	3.73

actively in a medium containing serum under a high concentration than in a medium made of a lower concentration of the same serum. On the contrary, if the same phenomena were caused by the progressive increase of a factor inhibiting the multiplication of fibroblasts, connective tissue cells should multiply more actively in a low than in a high serum concentration.

The value of these hypotheses was examined in a study of the growth of fibroblasts in high and low concentrations of the serum of

chickens 6 weeks, 3 months, 3 years, and 9 years of age. The media were made of fibrinogen suspension, serum, and Tyrode solution.



TEXT-FIG. 13. Variations of the rate of growth of fibroblasts in function of the serum concentration and of the age of the animal from which the serum was taken. The chart shows the modifications of the rate of growth in high and low serum concentrations of chickens 6 weeks, 3 months, 3 years, and 9 years old. The rate of growth in the lower serum concentration is considered equal to 100 per cent.

The serum concentrations used were generally 10, 50, and 70 per cent. When the serum of the older animal was used, the original fragment frequently contracted after a few hours. The growth was irregular and no accurate measurements could be taken. These changes

TABLE VII.

Rate of Growth of Fibroblasts in High and Low Concentrations of the Serum of a 3 Month Old Chicken, and in Tyrode Solution.

Experiment No.	Culture No.	Date.	Medium.		Medium.		Medium.		Medium.		Medium.	
			Serum 10 per cent; fibrinogen 20 per cent.	Serum 70 per cent; fibrinogen 20 per cent.	Serum 10 per cent; plasma 30 per cent.	Serum 70 per cent; plasma 30 per cent;	Tyrode solution 70 per cent; plasma 30 per cent.	Serum 70 per cent; plasma 30 per cent.	Serum 10 per cent; fibrinogen 20 per cent.	Serum 50 per cent; fibrinogen 20 per cent.	Serum 2.5 per cent; fibrinogen 20 per cent.	Serum 50 per cent; fibrinogen 20 per cent.
		1921										
1	20980	Apr. 21	3.89	4.04								
2	20981	" 21	2.79	2.66								
3	20982	" 21	3.05	3.55								
4*	21076	" 25			2.46	Shrunk-en.						
5	21077	" 25			2.58	2.25						
6	21078	" 25			4.55	4.31						
7*	21079	" 25					3.68	Shrunk-en.				
8	21080	" 25					2.32	1.02				
9	21081	" 25					4.0	2.81				
10	21100	" 26			3.70	2.57						
11	21101	" 26			3.40	2.05						
12	21102	" 26					3.70	2.57				
13	21103	" 26					3.40	2.05				
14	21167	" 29			4.50	3.47						
15	21168	" 29			3.65	2.84						
16	21169	" 29			4.37	4.33						
17	21170	" 29					6.43	2.64				
18	21171	" 29					3.05	3.20				
19	21172	" 29					3.70	1.40				
20	21227	May 2							1.28	1.33		
21	21228	" 2							1.09	1.03		
22	21229	" 2							1.62	1.26		
23	21230	" 2									1.79	1.74
24	21231	" 2									2.38	2.15
25	21232	" 2									1.73	1.93
26	21604	" 23							2.59	2.47		
27	21605	" 23							3.22	3.13		
28	21606	" 23							3.62	3.23		
29	21607	" 23							3.31	3.35		
Average.....			3.24	3.42	3.82	3.12	3.80	2.24	2.39	2.26	1.97	1.94

* Not included in average.

occurred earlier and were more marked when the serum of the 9 year old chicken was used. This peculiar action of the serum of the older animals rendered necessary the use of a serum concentration of 50 per cent. In other experiments 30 per cent plasma was substituted for 20 per cent fibrinogen suspension. The action of serum was also compared with that of Tyrode solution alone.

1. Serum of 6 Week Old Chicken.—The media contained respectively 10 and 50 per cent serum, and 20 per cent fibrinogen suspension. Five experiments were performed (Table VI). The differences in the rate of growth in the low and high serum concentrations were practically the same (Text-fig. 13). Therefore, the serum of the 6 week old chicken could be considered as deprived of a retarding as well as of an accelerating action on connective tissue cell proliferation.

2. Serum of 3 Month Old Chicken.—The media contained 2.5, 10, 50, and 70 per cent serum, and was completed in some experiments by 20 per cent fibrinogen suspension, and in others by 30 per cent plasma. There was no marked difference in the rate of growth in the low and high concentrations of serum (Table VII). It seemed, therefore, that the serum of a 3 month old chicken did not contain any accelerating factor. The slight retardation of the rate of growth observed in the higher serum concentrations was too small to allow any conclusion about the presence or absence of an inhibiting factor (Text-fig. 13). In a last series of experiments, the differences in the rate of multiplication of fibroblasts produced by 0 and 70 per cent serum were examined. The amount of growth obtained in 70 per cent serum was only 58 per cent of that observed in Tyrode solution.

3. Serum of 3 Year Old Chicken.—In seven experiments, the growth of fibroblasts in fibrinogen medium containing 10 and 50 per cent serum was studied. In other experiments, 30 per cent plasma was substituted for fibrinogen suspension. The growth was found to be constantly smaller in the media containing 50 per cent serum than in that containing only 10 per cent (Table VIII). In the media made of fibrinogen and serum, the amount of tissue produced in the 50 per cent serum was only 66 per cent of the amount in 10 per cent serum (Text-fig. 13). Since an increase of 40 per cent in the concentration of the serum in the culture medium decreased the amount of tissue produced by 34 per cent, the serum of the 3 year old chicken con-

tained, without any doubt, a factor which inhibited markedly the proliferation of fibroblasts *in vitro*.

TABLE VIII.

Rate of Growth of Fibroblasts in High and Low Concentrations of the Serum of a 3 Year Old Hen, and in Tyrode Solution.

Experiment No.	Culture No.	Date.	Medium.		Medium.		Medium.	
			Serum 10 per cent; fibrinogen 20 per cent.	Serum 50 per cent; fibrinogen 20 per cent.	Serum 10 per cent; plasma 30 per cent.	Serum 70 per cent; plasma 30 per cent.	Tyrode solution 70 per cent; plasma 30 per cent.	Serum 70 per cent; plasma 30 per cent.
		1921						
1	21001	Apr. 22			5.32	3.25		
2	21002	" 22			3.64	3.21		
3	21003	" 22			6.0	3.53		
4	21041	" 23			3.08	2.33		
5	21042	" 23			3.91	2.22		
6	21043	" 23			3.29	2.78		
7	21118	" 27			1.83	1.40		
8	21119	" 27			2.06	1.24		
9	21120	" 27			Spoiled.			
10	21121	" 27					3.13	1.48
11	21122	" 27					Spoiled.	
12	21123	" 27					2.18	1.24
13	21143	" 28			4.34	3.55		
14	21144	" 28			4.13	3.69		
15	21145	" 28			5.90	5.50		
16	21146	" 28			5.62	5.22		
17	21147	" 28			4.09	2.78		
18	21148	" 28			5.08	4.73		
19	21264	May 4	4.33	2.60				
20	21265	" 4	3.25	1.54				
21	21266	" 4	2.68	1.48				
22	21554	" 20	2.65	2.19				
23	21555	" 20	3.42	2.28				
24	21556	" 20	3.06	2.35				
25	21557	" 20	3.54	2.81				
Average.....			3.27	2.18	4.16	3.24	2.65	1.36

In a few experiments, the action of 70 per cent serum was compared with that of pure Tyrode solution, and under these conditions the serum also appeared to have a marked retarding action (Table VIII).

4. Serum of 9 Year Old Chicken.—In several experiments, no growth could be observed in the media containing 50 per cent serum because the tissue fragment contracted. However, in a few, the cultures remained normal and accurate measurements could be made (Table IX). The rate of growth in the high serum concentra-

TABLE IX.

Rate of Growth of Fibroblasts in High and Low Concentrations of the Serum of a 9 Year Old Cock.

Experiment No.	Culture No.	Date.	Medium.	
			Serum 10 per cent; fibrinogen 20 per cent.	Serum 50 per cent; fibrinogen 20 per cent.
		1921		
1*	21267	May 4	2.53	0
2*	21268	" 4	2.36	0
3*	21269	" 4	2.74	0
4	21304	" 6	1.82	1.03
5	21305	" 6	1.11	0.20
6	21306	" 6	1.60	0.80
7	21346	" 8	2.43	1.57
8	21347	" 8	3.24	1.46
9	21348	" 8	2.36	0.87
Average.....			2.09	0.99

* Not included in average.

tion was only 46 per cent of that observed in the low serum concentration (Text-fig. 13). When the medium contained 70 per cent serum, the tissue fragment practically always contracted and showed very little activity. The cells contained more fat granules in the higher concentrations of serum than in the lower. It appeared that the serum of an old animal determined certain changes in the tissue which could not be detected by mere measurement of the rate of proliferation.

The results obtained in the preceding experiments are represented in Text-fig. 13, in which the amount of growth in the high serum concentration is compared with that in the lower, which is considered equal to 100 per cent. In no case did the fibroblasts multiply

more actively in high than in low serum concentration. This demonstrated that in growing animals, the blood serum does not contain any accelerating factor for the proliferation of the fibroblasts. The higher concentrations of the serum of the older animals retarded the rate of multiplication markedly. There was no doubt, therefore, that age does not bring about the disappearance from the blood serum of an accelerating factor, but produces the increase of an inhibiting factor for the growth of fibroblasts.

III.

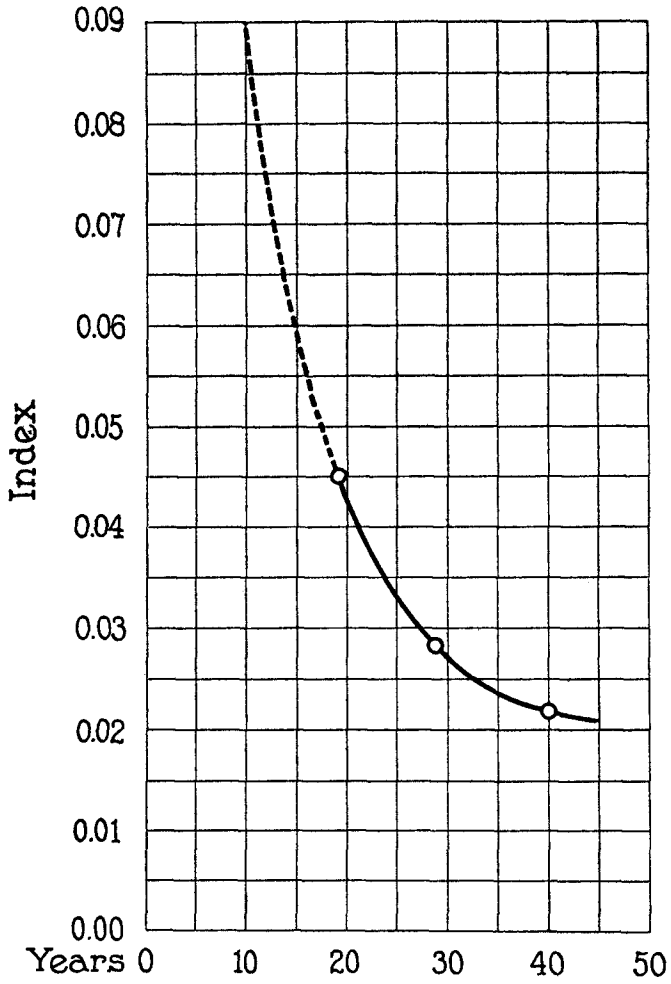
SUMMARY.

Pure cultures of fibroblasts displayed marked differences in their activity in the plasma of young, middle aged, and old chickens. The rate of cell multiplication varied in inverse ratio to the age of the animal from which the plasma was taken. There was a definite relation between the age of the animal and the amount of new tissue produced in its plasma in a given time (Text-figs. 1 to 10). The chart obtained by plotting the rate of cell proliferation in ordinates, and the age of the animal in abscissæ, showed that the rate of growth decreased more quickly than the age increased (Text-fig. 12). The decrease in the rate of growth was 50 per cent during the first 3 years of life, while in the following 6 years it was only 30 per cent. When the duration of the life of the cultures in the four plasmas was compared, a curve was obtained which showed about the same characteristics (Text-fig. 11). The duration of life of the fibroblasts *in vitro* varied in inverse ratio to the age of the animal, and decreased more quickly than the age increased.

As the differences in the amount of new tissue produced in the plasma of young, middle aged, and old chickens were large, the growth of a pure culture of fibroblasts could be employed as a reagent for detecting certain changes occurring in the plasma under the influence of age. But the method possesses the necessary accuracy only when it is used as has already been described,[3] and by technicians thoroughly trained in the details of its application.

A comparative study of the growth of fibroblasts in media containing no serum, and serum under low and high concentrations, was

made in order to ascertain whether the decreasing rate of cell multiplication was due to the loss of an accelerating factor, or to the in-



TEXT-FIG. 14. Curve showing the variations of the index of cicatrization of a wound 40 sq. cm. in area, in function of the age of the patient.

crease of an inhibiting one. In high and low concentrations of the serum of young animals, no difference in the rate of multiplication of fibroblasts was observed. This showed that the serum of an actively

growing animal did not contain any accelerating agent. The same experiments were repeated with the serum of a 3 year old and a 9 year old chicken. The medium made of a high concentration of serum had a markedly depressing effect on the growth, and this effect was greater in the serum of the older animal (Text-fig. 13).

The results of the experiments showed in a very definite manner that certain changes occurring in the serum during the course of life can be detected by modifications in the rate of growth of pure cultures of fibroblasts, and that these changes are characterized by the increase of an inhibiting factor, and not by the loss of an accelerating one. It appeared, therefore,that the substances which greatly accelerate the multiplication of fibroblasts and are found in the tissues[6] do not exist in the blood serum, or are constantly shielded by more active inhibiting factors. The curve which expresses the variations of the inhibiting factor in function of the age was compared with that showing the variations of the rate of healing of a wound according to the age of the subject. For wounds of equal size, the index of cicatrization, which expresses the rate of healing, varies in inverse ratio to the age.[7] The different values of the index of cicatrization of a wound 40 sq. cm. in area, taken from measurements made by du Noüy,[8] were plotted in ordinates, and the age of the subject in abscissæ (Text-fig. 14). The curve showed a decrease in the activity of cicatrization which resembled the decrease in the rate of growth of fibroblasts in function of the age of the animal. This suggested the existence of a relation between the factors determining both phenomena.

IV.

CONCLUSIONS.

1. Under the conditions of the experiments and within the limits of accuracy of the method, there is a definite relation between the rate of growth of a pure culture of fibroblasts, cultivated in plasma, and the age of the animal from which the plasma is

[7] du Noüy, P. L., *J. Exp. Med.*, 1916, xxiv, 451.
[8] du Noüy, P. L., *J. Exp. Med.*, 1916, xxiv, 463.

obtained, the rate of cell multiplication varying in inverse ratio to the age. A similar relation exists between the duration of life *in vitro* of the fibroblasts, and the age of the animal.

2. The variations in the rate of growth of a pure culture of fibroblasts may be used as a reagent of certain modifications occurring in blood serum under the influence of age.

3. The action of age on serum is characterized, not by the decrease of an accelerating factor for the multiplication of fibroblasts, but by the increase of an inhibiting factor.