THE INFLUENCE OF LECITHIN AND CHOLESTERIN UPON THE GROWTH OF TUMORS.*

By T. BRAILSFORD ROBERTSON, Ph.D., and THEODORE C. BURNETT, M.D.

(*From the Rudolph Spreckels Physiological Laboratory of the University of California, Berkeley.*)

In view of the marked influence of lecithin upon normal growth[1] we undertook to determine the influence of lecithin upon the growth of tumors; and since, in most instances, cholesterin acts in an antagonistic sense to lecithin, we decided to determine also the influence of cholesterin upon tumor growth.

METHODS.

Lecithin.—The lecithin employed was prepared in the following manner: The yolks of eggs were carefully washed in running water to free them from adherent whites. They were then broken and mixed, and an equal volume of a 10 per cent. solution of sodium chloride was added to them. The solution thus obtained was shaken up with twice its volume of ether until the mixture attained a thick oily consistency. It was then put aside over night in separatory funnels. In the morning the mixture had separated into sharply defined layers. The ether layer was removed and evaporated at room temperature to about one sixth of its volume, the lecithin was then precipitated by adding to this concentrated extract four volumes of acetone. The precipitate was allowed to settle in a tall glass cylinder, the greater part of the supernatant fluid was decanted, and the thick subnatant suspension of lecithin was collected upon a hardened filter. After washing this precipitate several times with acetone and allowing it to drain thoroughly, it was scraped off the paper into a glazed porcelain dish and dried over sulphuric acid in an incubator at 36° C. for over a week. The lecithin was then kept during the course of the experiment in a desiccator over sulphuric acid at room temperature. The product was a light brown mass which could be crumbled with difficulty and became somewhat pasty in consistency at temperatures of about 40° C.

In preparing the lecithin for injection into animals, a weighed amount was placed in a porcelain mortar and a corresponding volume of M/6 sodium chloride solution was heated nearly to boiling and a few cubic centimeters were poured

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[1] Danilewsky, B., *Compt. rend Acad. de sc.*, 1895, cxxi, 1167; King, H. D., *Biol. Bull.*, 1907, xiii, 40; Johnson, M. E., *University of California Publications in Zoölogy*, 1912, xi, 54.

into the mortar. The lecithin was softened by the hot water and could readily be macerated until it formed a thick pasty mass with the sodium chloride solution. The remainder of the sodium chloride solution, which had been allowed to cool to about 70° C., was then added little by little, and the mass was triturated thoroughly so as to obtain a uniform mixture. In this way fine and very stable emulsions of lecithin were readily prepared. They could be coagulated by boiling for several minutes or by adding salts of the alkaline earths. They were, however, employed for injections without further treatment.

Cholesterin.[2]—The usual difficulty was experienced in making stable suspensions. In the preliminary experiments we dissolved the cholesterin in a little hot alcohol and poured this solution into water. The suspensions thus formed, however, are not stable and contain numerous small crystals which block the hypodermic needle and render injection very difficult. Later it was found that acetone was much more suitable for this purpose than alcohol, and it had the further advantage that it could be readily driven off from the mixture by heating, and without injuring the stability of the emulsion. Such an emulsion does not deposit cholesterin even after standing at room temperature for over a month. Before this was ascertained, however, we found that very satisfactory emulsions could be obtained by triturating cholesterin in N/10 sodium oleate solution, and these were the emulsions employed throughout the second series of experiments. The weighed cholesterin was placed in a mortar, the sodium oleate solution was heated to boiling, and was then added little by little to the cholesterin, the whole mixture being carefully macerated and triturated to secure a uniform mixture. The emulsion thus obtained was heated just to boiling in an Erlenmeyer flask plugged with cotton, and was allowed to cool without removing the plug. Samples were withdrawn from time to time for use as needed.

HISTORY OF THE TUMORS.

We propagated the Flexner-Jobling carcinoma[3] by inoculation into the axillary region through four successive series of white rats, during which process the virulence of the tumor greatly decreased, the percentage of successful inoculations falling from 60 per cent. in the first series to 20 per cent. in the fourth series. The rats employed varied in age from half grown to adult, and were all obtained from local sources.

Preliminary Series.—In a preliminary series of experiments 101 white rats, obtained from local sources, were inoculated in the axillary region with portions of a tumor taken from the fourth series

[2] The cholesterin employed was Gruebler's purissimum crystallized or Kahlbaum's crystallized.

[3] Through the courtesy of Dr. Peyton Rous, of The Rockefeller Institute for Medical Research, we obtained in March of this year two specimens of the Flexner-Jobling carcinoma in white rats.

above mentioned. In all, there were twenty-four successful inoculations. These tumors grew extremely slowly, and of six which were kept as controls and not treated in any way, three underwent spontaneous degeneration and completely disappeared. The remaining three attained the average diameter of eleven millimeters only after five weeks of growth.

As our tumor appeared to have lost its virulence, the results obtained with these tumors, although confirmatory of the results subsequently obtained, were regarded as inconclusive.

For the second series of inoculations we therefore obtained two more specimens of the Flexner-Jobling tumor from the same source.

Second Series.—Sixty-one white rats were inoculated in the axillary region with portions of one of these tumors. The number of successful inoculations was 42, or 69 per cent.

At the same time sixty-four rats obtained from dealers in Chicago were similarly inoculated with portions of one of the control tumors from the preliminary experiment. The number of successful inoculations was 55, or 86 per cent.

Both sets of tumors grew rapidly, attaining average diameters of 15 and 11.9 millimeters respectively after nineteen days. The tumors obtained by inoculating local rats with the tumor supplied to us by Dr. Rous are designated as "local," while those which were obtained by inoculating rats obtained from Chicago with the tumor propagated here through five series of rats are designated "Chicago."

RESULTS.

In the preliminary experiments, as already stated, twenty-four animals with tumors were employed. The injections were started twenty days after inoculation. No accurate measurements of the tumors were made, but when the injections were begun their average estimated diameter lay between eight and eleven millimeters. Eleven animals received lecithin upon the 20th, 23d, 27th, 30th, 35th, 39th, 42d, and 46th days following the inoculation of the tumor, one cubic centimeter of 1 per cent. lecithin emulsion being injected directly into the tumors. Seven animals received cholesterin upon the twentieth and twenty-third days following the

inoculation, one cubic centimeter of 1 per cent. alcohol-cholesterin suspension being injected directly into the tumors. Six animals were kept as controls.

Even three days after the first injection it was noticed that the growth of the tumors in the animals that had received cholesterin had undergone a remarkable acceleration. Within a week they were double the size of the tumors in the control animals, and they maintained this lead throughout the experiment. Two spontaneous cures occurred after the forty-second day, whereas three of the tumors in the control animals underwent degeneration and had disappeared by the thirty-fifth day after inoculation.

The tumors in the animals treated with lecithin, on the contrary, grew more slowly than those in the normal animals and exhibited a greater proportion of spontaneous cures, for eight of the eleven tumors had degenerated and completely disappeared by the fiftieth day. On the fiftieth day the animals which still had tumors received two cubic centimeters of 2 per cent. lecithin emulsion. On the fifty-sixth day the average diameter of these tumors was twenty-two millimeters, while that of the tumor in the one survivor among the rats which had been treated with cholesterin was forty-four millimeters. After the fifty-sixth day the experiment was discontinued.

These results, although indicating a marked acceleration of tumor growth after the administration of cholesterin, and a possible retardation after the administration of lecithin, were inconclusive. The second series of experiments was therefore undertaken. The results of this series were decisive, and confirmed the tentative conclusions reached in the course of the preliminary experiments.

TABLE I.

Source of animal.	No. of controls.	No. treated with	
		1 c.c. of 2 per cent. lecithin emulsion.	1 c.c. of 3.9 per cent. cholesterin emulsion in N/10 sodium oleate.
Local	12	20	10
Chicago	12	30	13
Totals	24	50	23

The days on which injections of lecithin and cholesterin were given are shown in table II.

As already stated, ninety-seven animals ("local" and "Chicago") were inoculated with two tumors having different histories. No spontaneous cures occurred in any of the animals, and the tumors grew rapidly. The animals were apportioned without selection as shown in table I.

TABLE II.

Source of animal.	Days after inoculation.								
Local.....	19	21	24	26	28	31	33	35	38
Chicago.....	18	20	23	25	27	30	32	34	37

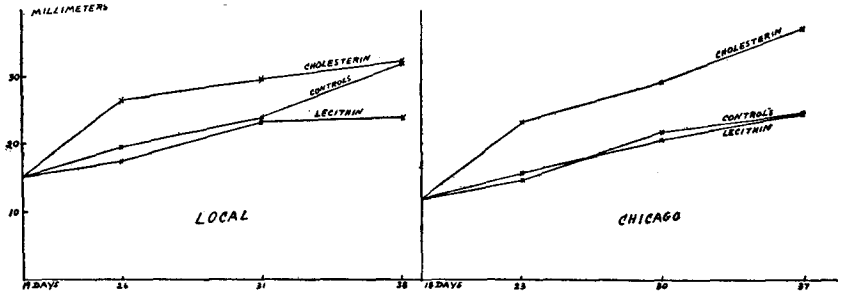
The tumors were measured through the skin in two diameters at right angles to each other on the 19th (18th), 24th (23d), 31st (30th), and 38th (37th) days after inoculation. When the tumors were small, the measurements were made with a pair of callipers. The mean of the longest and shortest diameters was recorded as the average diameter of any given tumor, and the average of these estimates gives the average diameter of the tumors in any given group of animals. This method of measurement was found to yield constant results, any ten unselected animals from either the local or the Chicago groups yielding, on the first day of measurement, the same figures for the average diameter within two or three millimeters. Consequently the average diameter of the tumors in the lecithin or cholesterin groups was considered to be that of the tumors in all the animals from the same source.

On the 24th (23d) day the lecithin animals were divided into two subgroups. On that day and thenceforward one half of these animals received lecithin suspended in M/6 sodium chloride and the other half received the same amount of lecithin suspended, however, in M/6 strontium chloride. The latter suspension was prepared by first suspending the lecithin in distilled water, and then adding sufficient concentrated strontium chloride solution to bring the final concentration up to M/6. The addition of the strontium chloride caused the lecithin to coagulate and form flocculi, which, however, did not prevent uniform mixture just before each injection, or impede the injection itself. Since strontium chloride renders lecithin insoluble, we employed a suspension of lecithin in strontium chloride solution, thinking that possibly lecithin injected

together with strontium chloride might stay longer within the tumors and thus exert its action, at least locally, for a longer period.

All the injections were made directly into the tumors.

The results obtained are given in table III and are also shown in text-figure 1.



TEXT-FIG. 1. Comparative growth of tumors treated with lecithin and cholesterin and untreated.

TABLE III.

Local Animals.

Treatment.	Average diameter of tumors in mm. after			
	19 days.	24 days.	31 days.	38 days.
Controls	15.0	19.8	24.0	31.9
Lecithin in M/6 sodium chloride	——	——	24.1	23.5
Lecithin in M/6 strontium chloride	——	——	22.6	24.5
Lecithin (inclusive)	15.0	17.6	23.4	24.0
Cholesterin	15.0	26.6	29.7	32.3

Chicago Animals.

Treatment.	Average diameter of tumors in mm. after			
	18 days.	23 days.	30 days.	37 days.
Controls	11.9	14.8	21.7	24.7
Lecithin in M/6 sodium chloride	——	——	20.0	24.0
Lecithin in M/6 strontium chloride	——	——	21.8	25.3
Lecithin (inclusive)	11.9	15.8	20.9	24.7
Cholesterin	11.9	23.3	29.2	37.2

It will be seen that the growth of the tumors in each group of animals was decidedly accelerated by the injections of cholesterin. This acceleration was, however, much more marked, especially in the local animals, in the first few days after the beginning of the treatments than it was later on, so that in the case of the local

animals, by the thirty-eighth day the size of the tumors in the controls almost equaled the size of the tumors which had been treated with cholesterin. On the other hand, scarcely any retarding action of lecithin is evident in the earlier stages of growth, but between the thirty-first and thirty-eighth days in the local animals the retardation due to lecithin was very evident. Table IV shows the comparative increase in growth.

TABLE IV.

Source of animal.	Treatment.	Increase in diameter in mm. between the		
		19th and 24th days.	24th and 31st days.	31st and 38th days.
Local.....	Controls	4.8	4.2	7.9
	Lecithin	2.6	5.8	0.6
	Cholesterin	11.6	3.1	2.6
Chicago.....	Controls	2.9	6.9	3.0
	Lecithin	3.9	5.1	3.8
	Cholesterin	11.4	5.9	8.0

No retardation of tumor growth due to lecithin is shown by the figures for the Chicago animals. As we shall see, however, the lecithin retarded markedly the growth of metastases in these animals, so that if we add metastatic to primary tumor growth the results obtained with these two groups of animals are identical in character.

It is evident from table III that the simultaneous administration of strontium chloride did not affect the action of lecithin.

Between the 31st (30th) and 38th (37th) days, four animals died in the Chicago lecithin group, one in the Chicago lecithin strontium chloride group, and one in the local cholesterin group. The tumors in these animals were measured on the days upon which death occurred, and were included in the estimates of the average diameters upon the 38th (37th) day. It was evident, however, from the poor condition of several of the animals, that more deaths might occur shortly and thus render comparison of the groups increasingly difficult and inaccurate.[4] Moreover, several animals had developed sinuses within the tumors and it was almost impossible

[4] Most of the animals that died or were in poor condition were found, upon post-mortem examination, to have pneumonic patches in the lungs.

to secure retention of the injected fluids within such tumors. Accordingly the experiment was terminated at this point, and a post-mortem examination of all the animals was carried out. Metastases were found in the lungs of many of the animals.[5] In table V the post-mortem findings are given.

TABLE V.

Source of animal.	Treatment.	No. of animals examined.	Broken down (scars opening upon the surface).		Metastases.	
			No.	Per cent.	No.	Per cent.
Local.....	Controls	11	3	27	3	27
	Lecithin	19	16	84	4	21
	Cholesterin	9	3	33	2	22
Chicago.....	Controls	8	0	0	4	50
	Lecithin	29	11	38	7	24
	Cholesterin	13	11	85	6	46

It is evident that in the Chicago animals lecithin greatly decreased the tendency to form metastases. This was evident in both groups of animals, but it was especially striking when the number and size of the metastases in the animals treated with lecithin were compared with those in the other animals.

When metastases were found in animals that had received lecithin they were small and few in number, usually consisting of one or two isolated spots in the lungs, while in all the animals treated with cholesterin in which metastases were found, and in three of the Chicago controls, the metastases formed dense masses throughout the greater part of the lung tissue. It may therefore be concluded that cholesterin greatly increases the extent of metastatic growth, while lecithin retards it.

Since in the animals treated with lecithin and in the local controls the metastases were few in number and small in extent on the thirty-eighth day, and since retardation of the primary tumor growth in the local animals was evident only between the thirty-first and thirty-eighth days, it would appear that the retarding action of lecithin occurs chiefly at a period of the growth of the

[5] We are indebted to Drs. F. P. Gay and G. Y. Rusk for examining these growths and confirming their metastatic nature.

tumors which roughly corresponds with the beginning of the metastatic stage.

CONCLUSIONS.

1. Cholesterin, whether suspended in dilute alcohol or in sodium oleate solution, when injected directly into tumors causes a marked acceleration both of the primary and of the metastatic growth.

2. The acceleration of the growth of the primary tumor by cholesterin is most evident in the premetastatic stage.

3. Lecithin, when injected in the form of an aqueous emulsion directly into tumors, diminishes the tendency to form metastases, retards the metastatic growth when it does occur, and in some instances also retards the primary growth.

4. The retardation due to lecithin is most evident in the metastatic stage.

5. Simultaneous injection of M/6 strontium chloride solution into the tumors does not appreciably affect the action of the lecithin.