FACTORS IN THE PRODUCTION AND GROWTH OF TUMOR METASTASES.*

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In the consideration of surgical operation in tumor cases the physician is often confronted with perplexing questions. In patients in which metastasis has already occurred will the growth of the secondary masses be accelerated by the removal of the primary tumors and will life thereby be shortened or prolonged? Do the procedures followed in the course of physical examinations or surgical operations increase or diminish the incidence of metastases? The variations in type presented by human tumors, the difficulty of subjecting a large number of similar cases to a uniform procedure, and the rarity of control unoperated cases with which to compare the treated, all serve to furnish inadequate grounds on which to base conclusions. Since such difficulties are avoided by employing tumors artificially propagated in animals, it may be of practical value to supplement clinical observations by experimentation with certain of these which have many characteristics in common with human tumors.

Investigations bearing on the occurrence of metastases in mice from which implanted tumors had been removed by operation have been made by Clunet.[1, 2] Of one hundred and forty-five mice which succumbed to implants of a certain strain of tumor (Tumor M), none showed metastases visible to the naked eye, although a post-mortem examination was made in each case. On the other hand, of eleven mice which were operated on for the removal of nodules of this tumor, five showed metastases. In the course of the propagation of another strain (Tumor F) only two of two hundred and thirty mice in which the growth of the implanted tumor resulted in death showed visible metastases on post-mortem

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examination. A series of mice bearing tumors of this strain were operated upon for the removal of the tumor with the result that twenty-four survived the immediate effects of the operation. In six of these the operation apparently resulted in a cure, while there was recurrence in the eighteen remaining. Nine of these showed visceral metastases visible to the naked eye at the time of death. Clunet thus found that metastases were more frequent in those mice which bore recurrent tumors for a considerable period of time following operation. The conclusion was reached that the development of metastases is not dependent upon the prolongation of life by operation, but is best explained by the theory of athrepsia advanced by Ehrlich [3] and defended by Apolant.[4, 5]

In undertaking the following investigation the object was not primarily to obtain data bearing on the question of athrepsia, but rather to determine if the operative procedures concerned in the removal of implanted tumors were instrumental in the production of metastases. Later on it was found possible by a further analysis of such operative procedures to determine the relative importance of certain of the component factors concerned in the production of metastases. For example, such a factor as the incision of a tumor should be considered apart from that of forcible manipulation, although both might be incident to operation. The results obtained make possible also a comparison of the rates of growth of metastases in operated and unoperated mice. It thus appears to be necessary to consider the question of the growth as distinct from that of the production of metastases, for, as it will be shown later, conditions which are most favorable for their growth do not produce them. A carcinoma which originated in the Japanese waltzing mouse was utilized in most of these experiments, although the propagation of the Ehrlich Stamme 11 mouse carcinoma and the Jensen rat sarcoma have yielded additional data.

The Japanese waltzing mouse tumor which has been propagated in this variety for a number of years, usually develops in one hundred per cent of waltzing mice on subcutaneous

inoculation, and, although it grows less rapidly than many of the transplantable tumors, when once established, it never undergoes retrogression. Metastases eventually develop in the lungs of a large proportion of the inoculated mice, but very rarely and only under certain conditions in other organs. It may be readily demonstrated by stained sections that these secondary nodules arise from emboli of tumor cells which lodge in the small blood vessels. In those mice in which there had been no intentional interference with the natural development of the implanted tumors, metastases visible to the naked eye have never been noted earlier than the thirty-ninth day after inoculation and in only one instance was a nodule noted as early. Lymph nodes even though they may be situated on the surface of large subcutaneous tumors which have grown continually by expansion are never invaded. The subcutaneous tissues of the mouse are loose, and the more or less uniform centrifugal growth of the tumor is not interfered with to any great extent by the surrounding structures. Following operations on these tumors the mode of growth may be somewhat modified, evidently as the result of trauma to the deeper tissues, together with a re-distribution of tumor cells, so that infiltration of the body wall occurs with extension to the peritoneal and pleural cavities.

EXPERIMENT 1.

March 29, 1912. Ten Japanese waltzing mice were inoculated subcutaneously on the right side with a bit of tumor from Mouse No. 3266. April 18, or twenty days later, nine mice presented tumors. The lot was then divided into two series, and the tumors excised from one series while the other served as a control. Recurrences in the operated series were excised in case the tumor did not appear to extend to the peritoneum or pleura.

EXPERIMENT 1.

Operated Mice.

Number.	Duration of Life After Inoculation.	Metastases.	Remarks.
3296	68 days.	O	Operations of April 18 and 26, May 10, and June 5, 1912. Died during fourth operation on recurrence.
3298	253 "	+	No recurrence appeared after first operation until Oct. 9, 1912, — 174 days later. This grew rapidly, killing mouse Dec. 7, 1912. Metastases minute but numerous.
3300	92 "	+	Operations April 18 and June 5, 1912. Recurrence. Post-mortem shows pleuritic effusion. Metastases larger than any previously seen.
3302	99 "	+	Operations April 18 and June 5, 1912. No recurrence. At death a mass of tumor measuring 9 x 7 x 6 mm. in lung.
3304	42 "	O	Operated April 18 and May 10, 1912. Died during second operation.

Control Mice.

3297	66 days.	.....	No data as to metastases. Viscera eaten by other mice.
3301	38 "	.....	No data as to metastases. Viscera eaten by other mice.
3303	67 "	+	One metastatic nodule in lung barely visible to the naked eye.
3305	54 "	+	Very minute metastases in lungs.

This preliminary experiment presents results which are in certain respects remarkable. In the propagation of this tumor mice have frequently lived for three and one-half months or longer after receiving implants, but none have presented metastases of more than 2.5 millimeters in diameter and rarely has this size been approximated. Metastatic nodules measuring four millimeters were found in Mouse No. 3300, which died ninety-two days after inoculation as the

result of lung involvement and pleuritic effusion, while the recurrent nodules beneath the skin were small and not ulcerated. Far more striking is the size of the metastatic mass found in Mouse No. 3302, in which the second operation was not followed by recurrence. This tumor of the lung presented the histological characteristics of the implanted tumor and cannot be regarded as a spontaneous primary tumor. Although operation for the removal of the subcutaneous tumor appears to have affected the rate of growth of metastases, it is to be noted that metastases did not occur in all of the operated mice. The numbers are, however, too small to furnish any indication as to the part played by operation in the production of metastases. It is evident that the removal of accessible masses of tumor by repeated operation tends to prolong life.

The greater part of the implanted tumors were next removed from a series of mice in order to determine if such operation had any appreciable effect on the production or growth of metastases, and also if it tended to prolong life.

EXPERIMENT 2.

May 10, 1912. Forty-two Japanese waltzing mice were inoculated subcutaneously on the right side with a bit of tumor from Mouse No. 3296. The mice were divided, on the basis of the size of the tumors which developed, into two comparative series. The tumors of one series were excised as thoroughly as possible, the operations occurring on June 5, 6, and 7, that is twenty-six, twenty-seven, and twenty-eight days after the inoculation. The animals were then kept until they died naturally and the number of metastases recorded.

EXPERIMENT 2.

Operated Mice.						Control Mice.				
Number.	Weight of Tumor Excised.	Metastases. Number.	Metastases. Maximum Diameter.	Duration of Life After Inoculation.	Remarks.	Number.	Metastases. Number.	Metastases. Maximum Diameter.	Duration of Life After Inoculation.	Remarks.
3346	.320	0	. . . . .	33 days.	Abscess, region of bladder.	3348	3	.5 mm.	68 days.	
3347	.200	11	1.5 mm.	74 "	Tumor sloughing.	3350	15	1.0 "	75 "	
3349	1.020	0	. . . . .	28 "	Died during operation.	3352	0	. . . . .	47 "	
3351	.390	10	4.0 mm.	67 "		3354	13	1.5 mm.	59 "	
3353	.540	7	.7 "	56 "		3356	4	1.0 "	54 "	
3355	1.130	17	4.2 mm.	68 days.		3357	5	1.5 mm.	62 days.	
3358	.760	1	1.5 "	64 "		3359	12	.4 "	58 "	
3360	.950	24	2.0 "	56 "		3361	3	.5 "	68 "	
3362	.520	8	2.5 "	97 "		3363	3	.5 "	64 "	Tumor sloughing.
3364	.900	11	2.0 "	66 "		3365	2	.4 "	57 "	
3366	.100	1	.5 mm.	83 days.		3367	19	2.3 mm.	75 days.	
3368	.330	30+	5.0 "	110 "	Metastases, large and numerous.	3369	22	1.9 "	66 "	
3370	.650	2	2.0 "	63 "		3371	1	.3 "	39 "	Earliest natural metastasis noted.
3372	1.220	16	7.0 "	101 "	Note large size of metastases.	3373	12	1.9 "	73 "	
3374	1.120	11	1.6 "	60 "		3375	4	1.2 "	57 "	
3376	.660	2	1.3 mm.	66 days.		3377	36	1.5 mm.	62 days.	
3378	1.265	0	. . . . .	27 "	Died on day of operation.	3379	8	2.0 "	61 "	
3380	.020	6	.5 mm.	75 "		3381	7	.5 "	56 "	
3382	1.600	6	2.0 "	75 "		3383	24	1.5 "	64 "	
3384	.865	27	4.0 "	70 "		3385	9	1.0 "	63 "	
3386	.650	22	1.3 "	66 "		3387	1	.5 "	42 "	
Total, 21	15.210	212	. . . . .	1,405 days.		Total, 21	203	. . . . .	1,270 days.	
Average,	.725	10.5	. . . . .	66⅔ days.		Average,	9.6	. . . . .	60½ days.	

Two of the operated mice died as the result of operation, while a third died of sepsis resulting from extension from a preëxisting abscess. Notwithstanding this loss, the average duration of life is somewhat greater in the operated series. There was recurrence in all the remaining operated mice and they, as well as the controls, eventually succumbed to the progressive growth of the tumor, and of the entire group all but one control mouse presented metastases. While there is no marked difference in the incidence of metastases in the two series, there is a definite difference in the size of the metastatic nodules which average much larger in the operated series. This might be explained on the ground either of earlier production, or of more rapid growth, or of a longer period of growth due to prolongation of life, through the removal of the subcutaneous tumor. A comparison of the metastases of operated with those of control mice which died after equal or slightly longer periods shows that the greater size of the metastases in the former is not dependent upon prolongation of life (see Fig. 1). Other operated mice (*i.e.*, Nos. 3368 and 3372) which outlived all the controls showed metastases far exceeding in size any ever observed in the natural course of the development of this tumor.

If, through operation, metastases are produced earlier than in a comparative series of non-operated tumor-bearing mice, this fact should appear in the following experiment, for at the time the mice were killed a large proportion had not yet developed metastases.

EXPERIMENT 3.

July 19, 1912. Four lots of Japanese waltzing mice were inoculated subcutaneously on the right side with bits of tumor from Mouse No. 3338. On August 19, thirty-one days later, the greater portion of the tumor was excised from one-half the animals while it was left undisturbed in the control series of equal number. On the same day bits of tumor from Mouse No. 3444 were implanted subcutaneously on the left side of both operated and control mice. All were killed forty-eight or forty-nine days after inoculation.

EXPERIMENT 3.

Improperly Operated Mice.					Control Mice.			
Number.	Metastases.	Weight of Tumor Excised.	Weight of Recurrence.	2d Implant.	Number.	Metastases.	Weight of Tumor.	2d Implant.
3388	3	.150	.025	.110	3389	0	.080	.020
3390	1	.005	.170	.020	3391	1	.670	.020
3392	0	.020	.025	.010	3393	0	.065	.010
3394	0	.040	.070	.010	3395	Eaten	by others.	.050
3396	4	.250	.070	.030	3397	0	.215	.020
3398	0	.015	.040	.020	3399	0	.610	.055
3400	1	.250	.650	.290	3401	0	.270	.020
3402	0	.005	.020	.020	3403	0	.925	.120
3404	0	.070	.170	.100	3405	5	.650	.070
3406	0	.020	.080	.040	3407	0	.150	.025
3408	0	.240	.470	.130	3409	0	1.840	.000
3410	1	.450	2.000	.340	3411	1	3.870	.000
3412	2	.070	.210	.010	3413	2	3.950	.000
3414	0	.200	.310	.140	3415	1	5.900	.000
3416	0	.270	.900	.185	3417	2	1.600	.120
3418	8	.080	.060	.070	3419	0	.500	.190
3420	0	.380	.100	.120	3421	Eaten	by others.	
3422	5	.100	.040	.015	3423	1	.760	.130
3424	0	.080	.050	.005	3425	Eaten.	.750	.150
3426	0	.040	.320	.270	3427	0	.630	.025
Total, 20	40%	2.735	5.780		Total, 17.	41%	23.435	
Average, ...		.136	.289	.096	Average, ...		1.302	.054

In considering the results of this experiment it is to be noted that in the third lot of mice the growth of the tumor is more rapid and that in the controls of this lot the second implant failed to persist in four out of five mice. In all four lots the average weight of the secondary implants after a period of eighteen days' growth in the operated mice is nearly twice that of the second implants in the controls. A more marked difference might be expected in case a more rapid growth should be obtained. The size of the secondary nodules is likewise usually greater in mice of the operated series, but the operation, although intentionally incomplete, has not, however, served to markedly increase the incidence of metastasis. This indicates that the greater size of the

metastases in operated mice may be dependent in a large proportion of cases not on their earlier production but on conditions resulting from the removal of the main portion of the subcutaneous tumor.

In operations in the three previous experiments, there was usually more or less tearing of the tumor mass and in the last, tumor tissue was intentionally left behind. In the following experiment the attempt was made to remove all tumor tissue and at the same time to avoid tearing or incision of the mass as well as unnecessary pressure.

Experiment 4.

July 19, 1912. Twenty Japanese waltzing mice were inoculated with bits of tumor from Mouse No. 3338. On Aug. 19, 1912, thirty-one days later, all presented tumors. The tumors of one-half this number were then removed as completely as possible by surgical operation, care being taken to avoid manipulating and pulling on the tumor. Of the ten remaining mice which served as controls, five possessed at this time tumors slightly larger, and five slightly smaller than those of the operated mice with which they were paired. Both operated and control mice were killed fifty-five days after inoculation and the lungs in each case examined for metastases.

Experiment 4.

Operated Mice.				Control Mice.		
Number.	Weight of Tumor Excised.	Weight of Recurrence at Death.	Metastases.	Number.	Weight of Tumor at Death.	Metastases.
3428	.070	.650	O	3429	.220	+
3430	.170	.005	O	3431	.200	+
3432	.150 (Died during operation.)			3433	1.300	+
3434	.280	.020	O	3435	.960	+
3436	.210	.000	O	3437	1.080	+
3438	.350	.055	O	3439	1.900	+
3440	.120	.010	O	3441	1.500	+
3442	.085	.220	O	3443	1.700	+
3444	.580	.020	+	3445	.570	O
3446	.140	.005	O	3447	.705	+
Total, 9	2.155	.985	1 or 11%	Total, 10	10.135	9 or 90%
Average ..	.215	.109		Average ..	1.013	

The difference in the incidence of metastases in the two comparative series is marked. Although recurrence appeared in eight of the nine mice which survived, with two exceptions these were relatively small. A careful operation has here prevented metastasis in eight out of nine mice and it is probable that by a similar procedure applied to the recurrences the tumor might eventually have been completely eradicated in a number of these. Whereas preceding experiments having yielded data concerning the growth of metastases, these results have a definite bearing on their production. From the incidence of metastases in the control series, it is evident that the stock of mice used was unusually favorable for their formation, and furthermore the time factor was equal in both operated and control mice, with the possible exception of one (No. 3436) in which the tumor did not reappear after operation. The minute size of the residuum of the subcutaneous tumor in the period immediately following the operation as compared with either the amount of tumor then growing in the control series or that left in the operated mice of Experiment 3 serves to explain the absence of metastases. In fact in the amount of tumor present is found the only variant condition, and on this basis it may be reasonably postulated that, other conditions being equal, the chances of the production of metastases will increase with the increase in the size of the primary tumor.

In investigating further the factors concerned in the production of metastases, it appears important to consider mechanical force, as illustrated by manipulation or massage of the primary tumor, apart from surgical operation. Although the exercise of unnecessary manipulation or force in the physical examination of tumors has long been condemned on theoretical grounds, I am unaware of the experimental demonstration of the results of procedures of this sort.

EXPERIMENT 5.

Sept. 12, 1912. Twenty-two Japanese waltzing mice were inoculated subcutaneously on the right side with bits of tumor from Mouse No. 3443.

On October 9, twenty-seven days later, these mice were separated on the basis of the size of the implanted tumor into two comparative series, and the tumors of one series were manipulated and massaged between the thumb and forefinger. Considerable pressure was exerted, and, although definite rupture of the tumor occurred in several instances, this was avoided as far as possible. The tumors of this series were afterward repeatedly subjected to massage, which was done twenty-nine, thirty-three, thirty-four, and thirty-five days after the inoculation. The mice of both series were killed forty-six days after inoculation at the threshold, so to speak, of the usual period of metastasis development, and the organs, more especially the lungs, were examined for metastases.

EXPERIMENT 5.

Tumor Massaged Mice.			Control Mice.		
Number.	Weight of Tumor.	Metastases.	Number.	Weight of Tumor.	Metastases.
3450	.060	O	3452	.100	O
3453	.750	O	3454	.270	O
3455	.000 (Wholly degenerated.)	O	3458	2.400	+ (Six.)
3456	2.200	+ (Numerous.)	3459	2.300	O
3457	2.400	+ (Three.)	3460	.110	O
3462	2.000	O	3461	2.900	+ (One.)
3464	1.000	+ (Two.)	3465	2.400	O
3466	.850	+ (Two.)	3467	1.080	+ (One.)
3468	1.350	+ (Three.)	3469	.120	O
3470	2.300	+ (One.)	3471	.900	O
3472	.700	+ (Two.)	3473	1.250	O
Total, 11	13.610	7 or 63⅔%.	Total, 11	13.830	3 or 27⅓%.

In the first lot of twelve mice the metastases are evenly distributed between the massaged and the control series, but in the second lot metastases are much more frequent in the massaged.

In the two lots combined metastases are more than twice as frequent in the massaged mice. They are notably absent in mice bearing the smaller tumors showing again the importance of the size of the primary growth in the production of metastases. The minute size of many of the metastases together with the occurrence of inflammatory lesions in these series made it necessary to confirm the naked eye diagnosis by the examination of stained sections of all questionable nodules.

EXPERIMENT 6.

Oct. 28, 1912. Twenty-three Japanese waltzing mice were inoculated subcutaneously on the right side with bits of tumor from Mouse No. 3458. (One mouse, No. 3496, died shortly after massage.) After dividing this lot into two comparative series of ten each on the basis of the size of the tumor presented — on November 15 and 18, eighteen and twenty-one days respectively after inoculation, the tumors of one series were forcibly manipulated and massaged between thumb and forefinger. The other series was intended to serve as a control by which to estimate the effect of the manipulation of the subcutaneous tumor with respect to the formation of metastases. Both series were killed thirty-six days after the inoculation (or fifteen days after the last massage), the subcutaneous tumors weighed, and the organs examined for metastases.

EXPERIMENT 6.

Tumor Massaged Mice.			Control Tumor Mice.		
Number.	Weight of Tumor.	Metastases.	Number.	Weight of Tumor.	Metastases.
3475	1.050	+ (Twenty or more.)	3474	1.700	O
3477*	2.000 (approximately).	+ (Numerous.)	3476	.200	O
3480	1.070	+ (Two.)	3478†	1.090	O
3482	1.170	+ (Twelve or more.)	3481	.250	O
3484	.500	+ (Innumerable.)	3483	.220	O
3486	Eaten by	other mice.	3485	(Killed 40 days after inoculation with large tumor. No metastases.)	
3488	.200	O	3487	.850	O
3490	.900	+ (Two.)	3489	1.170	O
3492	1.150	+ (Five.)	3491	.400	O
3494	.540	+ (Three.)	3493	.750	O
3495‡	.450	O	3497	1.300	O
Total, 10	9.030	8 or 80%	Total, 10	7.930	0 or 0%
Average...	.903		Average ..	.793	

*Died thirty-five days after inoculation.
†Died thirty-three days after inoculation.
‡Massaged once only, twenty-one days after inoculation.

Since no metastases have been observed in the natural development of this tumor before the thirty-ninth day after inoculation, the results of massage are even more marked than in Experiment 5.

In order to see whether the emboli of tumor tissue may be demonstrated in the lung soon after massage, stained sections of the lungs of several mice were studied.

EXPERIMENT 7.

Dec. 7, 1912. Eight Japanese waltzing mice received implants of tumor beneath the skin of the right side. Sixteen days later the tumors in one-half of this number were massaged while the others served as controls.

EXPERIMENT 7.

Number.	Series.	Killed.	Stained Sections of Lungs Show
3504	Massaged.	At once after massage.	No demonstrable tumor emboli.
3505	Control.	At once after massage.	" " " "
3506	Massaged.	24 hours after massage.	" " " "
3507	Control.	24 hours after massage.	" " " "
3508	Massaged.	3 days after massage.	Numerous minute masses of tumor growing out from small blood vessels.
3509	Control.	3 days after massage.	No demonstrable metastases.
3510	Massaged.	41 days after massage.	Six or more metastases, the largest measuring 2 mm. in diameter.
3511	Control.	41 days after massage.	No metastases visible to naked eye.

Although the number of mice employed was small, metastases were demonstrated in the lungs three days after massage and only nineteen days after inoculation. This observation served as a basis for an attempt to effect still earlier metastasis, and with the intention of favoring the growth of the

secondary nodules as much as possible in the following experiment, the primary tumor was excised from one of the series of mice following its massage.

EXPERIMENT 8.

Feb. 26, 1913. Three lots comprising in all thirty-six Japanese waltzing mice received implants of tumor which grew progressively in every instance. Each lot was divided into three series, and in the first the tumor was left untouched for control purposes, in the second the tumor was massaged for a short time at first very gently nine, ten, twelve, thirteen, and fifteen days after the inoculation, while in the third series the tumor after being subjected to an identical massage treatment was excised on the day following the last massage, sixteen days after inoculation.

Experiment 8.

Treatment of Tumor.									
None (Controls.)			Massaged 9, 10, 11, 13, and 15 Days After Inoculation.			Excised 16 Days After Inoculation Following Massage as in Preceding Series.			
Number.	Weight of Tumor at Death.	Metastases.	Number.	Weight of Tumor at Death.	Metastases.	Number.	Weight of Tumor.		Metastases.
							Excised.	At Death.	
3645	1.520	O	3646	2.730	O	3647	.250	.100	O
3648	.480	O	3649	.960	O	3650	.350	.140	O
3651	.650	O	3652	.530	O (Pregnant.)	3653	.140	.400	O
3654	.790	O	3655	.330	O	3656	.180	.070	O (Pregnant.)
3657	1.300	O	3658	1.550	+ (Two.)	3659	.130	.060	+ (Numerous, minute.)
3660	.840	O	3661	.940	O	3662	.330	.190	+ (Two.)
3663	1.300	O	3664	1.570	+ (Pregnant.)	3665	.430	.260	+ (Four.)
3666	1.150	O	3667	.300	+ (Three.)	3668	.470	.100	+ (Five.)
3669	.330	O	3670	1.890	+ (Five.)	3671	.100	.550	+ (Innumerable.)
3672	2.550	O	3673	1.650	O	3674*	.630	1.900	+ (In liver and kidney.)
3675	.300	O	3676	.500	O	3677	.140	.490	+ (Sixteen.)
3678	.280	O	3679	.660	O	3680	.100	.310	O
Total, 12	11.490	0 or 0%	Total, 12	13.910	4 or 33⅓%	Total, 12	3.250	4.570	7 or 58⅓%
Average...	.958		Average ...	1.160		Average ...	.271	.381	

* Distribution of metastases to be explained by infiltration of body wall with primary tumor.

The results obtained in this experiment furnish further proof of the importance of mechanical force in metastasis formation. The absence of metastases in all series of the first lot of mice (Nos. 3645 to 3656 inclusive) is difficult to account for since they were of the same stock and of approximately the same age as those of the other two lots. That their early appearance in mice of the third series is due to more rapid growth as a result of the removal of the greater part of the subcutaneous implant appears possible. The lung nodules in this series are on an average slightly larger than those in the series of massaged mice, with the exception of Mouse No. 3664, in which they are of still greater size as well as numerous. That the pregnancy of this mouse, which evidently commenced about twelve days after inoculation, should influence the growth of metastases to such a degree would appear remarkable, for there is no proportionate growth of the primary tumor which does not exceed in size others of the series. It is not improbable that the first massage served to set free tumor cells in this mouse so that the resulting metastases grew for a slightly longer period than was the case with others.

The Ehrlich Stamme 11 tumor, a transplantable carcinoma of the common mouse which has been propagated for many years in this laboratory, does not produce metastases. Neither have secondary tumor nodules been observed in the propagation of the Jensen rat sarcoma during the past year. Attempts to produce metastases artificially by massage of the primary implanted tumors of these two strains have thus far proved unsuccessful.

In the theoretical consideration of the subject of the development of metastasis it becomes necessary to make a distinction between the factors concerned in their growth (in a restricted sense) and those concerned in their production. Thus although conditions favorable to growth are necessary to the development of metastases, such conditions do not of themselves produce them. It is probable that by a similar analysis of the condition of tumor-immunity into its component factors, much of the existing confusion

might be avoided. Notwithstanding the frequency with which operations for the removal of implanted tumors have been performed (Schöne,[6, 7] Uhlenhuth, Haendel, and Steffenhagen,[8, 9] Apolant (loco. cit.), Russell,[10] Walker and Whithingham [11]), I have been unable to find any reference other than Clunet's bearing on the production of metastases by procedures of this sort. With respect to an increase in the rate of growth of embolic deposits of tumor tissue as the result of the removal of the greater part of the primary growth, the results of Experiments 1, 2, 3 are in accord with Clunet's interpretation of his findings. In order to account for the development of metastasis on the basis of athrepsia, however, he assumes, without furnishing any evidence in support from his own material, that emboli of tumor cells are commonly present in the lungs of mice in which, under natural conditions, gross metastases occur with extreme rarity. Clunet presents no parallel series of unoperated mice, by which to control — with respect to the period of time elapsing between the inoculation and the appearance of metastases — his operated series, but merely compares the results obtained in the latter with those obtained at large in the propagation of the tumors. The possibility of tumor cells being liberated during operation is summarily dismissed with the assumption that this is continually taking place.

In the present series of experiments it has been shown that metastases may develop notwithstanding the complete removal of the primary tumor (see Experiment 1, Mouse No. 3302). Clunet's invariable failure to obtain secondary nodules in those mice in which there was a complete removal of the primary growth indicates, therefore, that factors other than athrepsia were, in his experiments, instrumental in the production of visible metastases, while the importance of forcible manipulation is shown in the results of the last four experiments with the Japanese waltzing mouse tumor.

Summarizing it is evident that the production of metastases is dependent upon a number of determinable factors, of which the biological character of the tumor tissue is of first

importance. Secondary deposits are rarely or never observed in certain propagated tumors while they are frequent in others, and this is also true for the various types of spontaneous tumors. It is probable that the mechanism by which tumor cells are set free in the circulation is dependent to a great extent upon the structural character of the tumor and the peculiarities of its growth.

Time is also an important factor. A tumor of a given strain growing continuously for a single month in an inoculated animal is less likely to furnish metastases than one growing for a longer period. This is shown by the incidence of the secondary nodules in mice taken at various intervals after the inoculation of tumors which metastasize.

That the size of the primary tumor enters into the question of the incidence of metastasis is indicated by the results obtained in Experiment 4. The reduction in the number of metastases as the result of incomplete though careful operation is here marked, notwithstanding that the observation was made after the tumor tissue had been present, with one exception in which there was no recurrence, for the same length of time in both operated and control mice. The dissemination of tumor cells apparently takes place more readily after the tumor has attained considerable size, and is infrequent except under unusual conditions during the earlier growth of the implant.

By the application of intermittent pressure such as may be accomplished by massage or gentle pinching, it is possible to produce metastases experimentally with the tumor of the waltzing mouse. It may be contended that, by such means, larger emboli of tumor cells are produced so that the metastases become visible to the naked eye earlier than those derived from smaller emboli set free correspondingly early in control tumor bearing mice. If this were true to any great extent, the distribution of the deposits would be different and larger vessels would be blocked in the massaged mice, but this is not borne out by observation, since the metastatic nodules are located at or near the surface of the lung in these as well as in control mice, while by histological study

minute masses of tumor were found only after the lapse of three days from the time of the massage, and these were found in the smaller arterial branches and capillaries of the lung.

The medium furnished for the growth of the tumor varies with the individual mouse. An analysis of the tables accompanying Experiments 5, 6, and 8 will show a greater frequency of metastases in those mice which present the larger tumor after a given period of growth. This may indicate a more favorable soil for the growth of tumor emboli, but it is more probable that the greater size of the primary tumor favors earlier dissemination. Neither of these possibilities give support to the view that the formation of metastases is based on conditions to be included under athrepsia. The only possible action of athrepsia would be of a negative nature in the prevention of the growth of emboli of tumor cells through lack of substances necessary for their nutrition. Distinctly against this is the absence of metastases in a series of mice from which the primary tumor was removed just prior to the time at which dissemination of the tumor cells usually occurs (Experiment 4).

The pre-metastatic period. — The hypothesis has been suggested by Sticker[12] and adopted by Gay[13] that the failure of tumors to metastasize during the early period of their growth is dependent upon a phase of "active resistance." According to these investigators second implants of tumors made during this period fail, while those made later during the "metastatic" period are successful. Second inoculations with Japanese waltzing mouse tumor are successfully made during the pre-metastatic period, although the occurrence of metastases closely corresponds in point of time with that observed in the Flexner-Jobling rat tumor; but those made early grow more vigorously than those made later after the first implant has acquired considerable size. The dissemination of tumor cells may be accomplished by massage of the primary tumor and by this measure the pre-metastatic period may be greatly reduced. Secondary

tumor nodules which are produced by massage done either on or prior to the fifteenth day are macroscopically visible thirty days after inoculation. It is apparent (Experiment 6) that the metastatic nodules attain sufficient size in from fifteen to eighteen days as to be visible to the naked eye. Massage appears to be more efficient if performed relatively early (eighteen to twenty-one days) than when done just prior to the period of natural metastasis. The pre-metastatic period is, therefore, explainable on other grounds than that of a period of "active resistance" — chiefly by the fact that the opportunities for dissemination are increased proportionately with the length of the period of growth and with the size of the primary tumor.

The rate of growth of metastatic nodules is influenced by either the partial or the complete removal of the primary implants of this tumor, so that application for the principle of athrepsia may possibly be found in the growth rather than in the production of metastases. That athrepsia is a determining factor even with the reference to rate of growth is contraindicated by certain of Russell's [10] carefully planned and comprehensive experiments with reference to active resistance to tumor growth. He finds that second implants in a series of mice bearing large tumors may exceed by actual weight control implants in a series of normal mice. While it is stated earlier in his paper that all experiments were adequately controlled, the significance of these results is such that exact data especially with regard to age and stock of each series of mice should be furnished. Other investigators state that second implants grow more slowly or not at all. The size attained by the metastases in the operated mice of Experiments 1, 2, and 3 already discussed appear to justify the further consideration of athrepsia with respect to rate of growth, however inadequate it may prove with respect to tumor immunity.

It has been noted during the course of these experiments that cachexia develops in the controls earlier than in the operated mice and, since this impairment of the entire organism may involve factors other than the lack of specific food

substances which influence tumor growth, the problem is not as simple as it would at first appear.

The results obtained in this investigation find practical application in the management of tumor patients. They are of such character that every physician should realize the irreparable harm which may result from the manipulation of malignant tumors in their early development. Although the present observations are made on a tumor with which dissemination usually takes place by way of the blood stream, it seems reasonable to expect similar results with human tumors which become disseminated by way of the lymphatics. The course of procedure to which the patient is frequently subjected, as I have repeatedly observed — the palpation of the mass in question in repeated physical examinations, the violent scrubbing often employed in preparing the field of operation — is almost identical with that which I have employed for the experimental production of metastases. It would be of advantage to the patient if each questionable tumor of the breast, for example, could be regarded as a high explosive, the least manipulation of which should be absolutely avoided both prior to and during the operation. It is not improbable that by this means metastasis and extension beyond the field of operation could be prevented and the percentage of cases cured by operation increased. From the point of view of metastasis it would appear from these results much less serious to cut into a tumor than to exert pressure upon it, although the effect of the distribution of tumor tissue throughout an extensive operation is quite generally understood. It is not improbable that the removal of a tumor of large size from which metastasis has already occurred results in a more rapid growth of the secondary deposits. This should not weigh too heavily, however, in considering palliative operations of this sort, for internal tumors not being under the direct observation of the patient furnish a less constant source of apprehension, and the removal of a large external tumor may make the case much easier for the physician to manage.

RECAPITULATION OF THE RESULTS OF EXPERIMENTS WITH THE WALTZING MOUSE TUMOR.

Operations incomplete but involving the incision of implanted tumors do not increase the incidence of metastases, but these grow more rapidly as the result either of an increase in the amount of food material made available by the removal of a large mass of tumor tissue elsewhere (athrepsia) or of the elimination of the element of cachexia and improvement of the physical condition which almost invariably occurs.

Radical operations involving the removal of all the tumor — except minute masses which subsequently come into evidence along the path of the inoculating trochar — if performed just prior to the period in which metastasis commences, result in a temporary freedom from this complication even though recurrence commonly occurs.

Metastasis may be artificially produced by the manipulation and massage of the implanted tumor. This is accomplished as readily during the early development of the tumor as in the period in which metastasis naturally occurs.

The production of metastases is dependent on certain demonstrable factors — the biological character of the tumor, the duration of its growth, the size of the primary mass, possibly peculiar conditions furnished by the host tissues, and, under artificial conditions, forcible manipulation.

The so-called " pre-metastatic period " is better accounted for by an absence of the conditions necessary for the dissemination of tumor cells than by a phase of " active resistance " during which the further development of tumor emboli is prevented. By the early artificial dissemination of tumor cells by the manipulation of the primary tumor, this period may be greatly shortened.

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DESCRIPTION OF PLATE XVIII.

FIG. 1. — Large metastatic nodules in lung of Mouse No. 3300. Recurrent tumor shown attached to skin of right side. This mouse died ninety-two days after inoculation. Operations twenty days and sixty-eight days after the inoculation. (See Experiment 1.)

FIG. 2. — Mouse No. 3368 which died one hundred and ten days after inoculation. Operation twenty-eight days after inoculation. The tumor has to a large extent replaced the lungs and fills a greater part of the thorax. The recurrent tumor nodules are attached to the skin dissected back from the right side of the mouse. (See Experiment 2.)

FIG. 3. — The upper row of lungs represented in this figure were from the five mice of the operated series of Experiment 2, and those of the lower row were from the five mice of the control series, showing the

largest metastatic nodules in each respective series up to the seventy-fifth day.

OPERATED MICE.

No. 3360.	No. 3370.	No. 3351.	No. 3355.	No. 3384.
56 days.	63 days.	67 days.	68 days.	70 days.

CONTROL MICE.

No. 3354.	No. 3379.	No. 3348.	No. 3373.	No. 3367.
59 days.	61 days.	68 days.	73 days.	75 days.

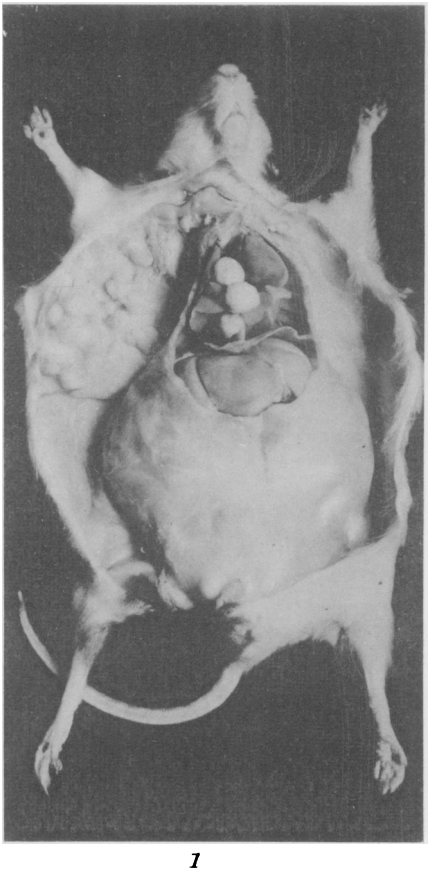
Although with the exception of the second pair, the duration of life is greater in the controls selected, the metastases are in every instance smaller than in the corresponding operated mice. The difference is more marked in the later development, sixty-seven to seven-five days, than in the earlier.

FIG. 4. — The lungs of Mouse No. 3372 showing large nodules and a greater part of one lobe infiltrated with tumor. Death one hundred and one days after inoculation. Operation twenty-eight days after inoculation. (See Experiment 2.)

FIG. 5. — The lungs of Mouse No. 3510 which was massaged sixteen days after inoculation and killed forty-one days after inoculation. Secondary nodules are much larger than those ordinarily found in the control mouse after this period. The control for this mouse showed no metastases. (See Experiment 7.)

FIG. 6. — The lungs of Mouse No. 3664 infiltrated throughout with metastases. This was taken thirty days after the inoculation, and the early appearance of the metastases is attributable to the massage of the subcutaneous tumor which was done on the tenth, eleventh, thirteenth, fourteenth, and sixteenth days after inoculation. (See Experiment 8.)

FIG. 7. — Microscopic metastases near pleural surface (below) of Mouse No. 3508 killed three days after the massage of the primary tumor which was done sixteen days after the inoculation. (See Experiment 7.)



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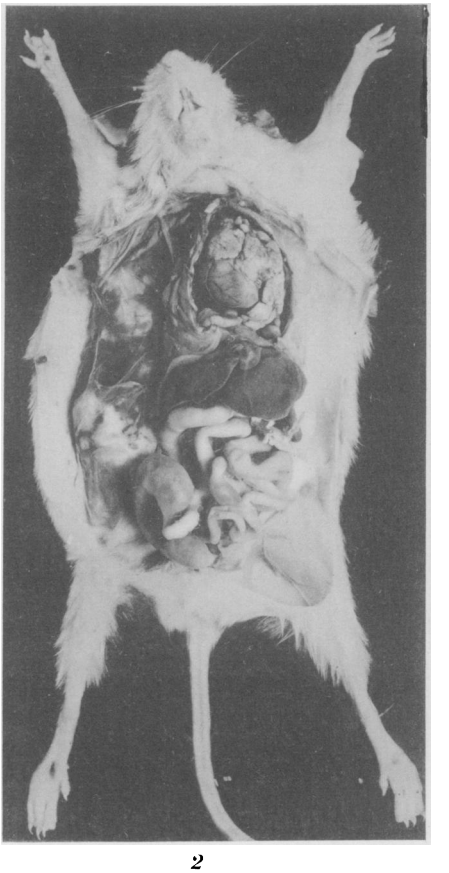

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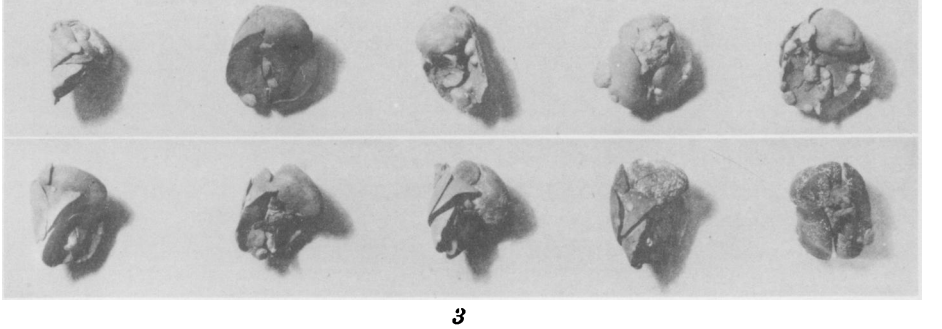

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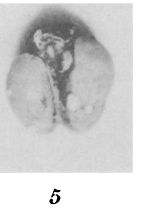

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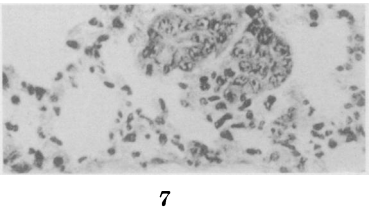

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