

of the tumor.¹ The second series of 202 young adult male rabbits from 19 standard breeds was reported by the author in 1938.² The animals in both series received a single intratesticular injection of 0.3 to 0.5 cc of a saline emulsion of rabbit tumor tissue. In the first series 114 and in the second series 137 animals presented gross metastases at necropsy. The postmortem technic was the same in both series, but there were slight differences in the particular muscles, bones, and distant nodes selected for examination.

The distribution of metastases in the first and second series is presented in Table I for those sites in the body concerning which comparable data are available. The data for regional and distant metastases are segregated. Both the actual and the expected values are presented.

Neither by local extension nor hematogenous spread was there a significant variation between the actual and the expected values for the distribution of metastases. There was some evidence, furthermore, that the experimental error in the 2 series was unusually small (chi-square = 8.53, $n = 17$, $P = 0.95$). The seemingly low experimental error in the observations is borne out by the coefficient of correlation between the 2 series of +0.9828 ($n = 16$, $P = 0.001-$), a very high and significant value. The regression line is linear.

It was therefore concluded that the Brown-Pearce tumor had undergone no change in the relative distribution of metastases during 15 years of transplantation into the testicle. The distribution of metastases, furthermore, was found to be constant and characteristic for each organ.

10365

Distribution of Metastases in the Brown-Pearce Tumor. III. Effect of Homologous Material.*

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It has been shown that a single injection of a saline emulsion of 0.05-0.1 cc of Brown-Pearce tumor tissue which has been kept

¹ Pearce and Brown, *J. Exp. Med.*, 1923, **38**, 347.

² Casey, *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 228.

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frozen for 10 days or longer in an anaerobic state will render rabbits susceptible to subsequent transplantation with the tumor.¹ The injection of this homologous material increases (a) the incidence and volume of the primary tumor growths, and (b) the incidence, number, and volume of the metastases, with a consequent decrease in the longevity and an increase in the mortality of the animals. The material, when injected, produces no obvious lesions, nor does it visibly affect the health of the animal. It can be desiccated, passed through a Berkefeld "V" filter,² and inactivated by heating to 55°C.³ It differs biologically from the spreading factor described by Duran-Reynals.⁴

It has been shown that the relative distribution of metastases in this tumor is constant and characteristic following intratesticular inoculation, and remains unaffected by long transplantation into various breeds of rabbit.⁵ The present paper concerns the possible effects of the injection of the homologous material in relation to the relative distribution of metastases.

Two groups of rabbits were used. The first consisted of 202 young adult male rabbits from 19 standard breeds, which were injected with the tumor alone.⁵ The second consisted of 44 young adult male rabbits of pure and mongrel stocks, which received a single injection of 0.3 cc of the homologous material into one testicle 2 weeks before inoculation with the tumor. Both groups of animals received a single unilateral intratesticular injection of 0.3 cc of a saline emulsion of fresh Brown-Pearce tumor. The variations of breed in the 2 groups were not considered important, since this factor has been shown not to affect the relative distribution of metastases.⁶ Complete necropsies were performed in the usual manner¹ on the animals which died spontaneously, and at the end of 2 months on all the animals which had survived.

The number of instances in which metastases were found in various sites are summarized in Table I, which presents both the actual and the expected values. For facilitation of the statistical analysis it was convenient to assume that the relative distribution of metastases was constant and characteristic in the 2 series, and that variations from the expected values could be attributed to ex-

¹ Casey, *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 816; *Am. J. Cancer*, 1934, **21**, 760.

² Casey, *Am. J. Cancer*, 1936, **26**, 276.

³ Casey and Moragues, in press.

⁴ Casey, *Arch. Path.*, 1937, **23**, 741.

⁵ Casey, *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 228.

⁶ Casey, *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 230.

TABLE I.
Instances of Metastasis in Various Organs After Intratesticular Inoculation of the Brown-Pearce Tumor.

Site	Actual Values			Expected Values			$\times 2/m$		
	Controls	Treated	Totals	Controls	Treated	Totals	Controls	Treated	Totals
Tunic extension	53	28	81	57.0	24.0	81	.28	.67	.95
Primary cord	89	35	124	87.3	36.7	124	.03	.08	.11
Opposite "	34	22	56	39.4	16.6	56	.74	1.76	2.50
Pelvis and bladder	72	33	105	73.9	31.1	105	.04	.12	.16
Para-aortic tissues	92	39	131	92.2	38.8	131	.00	.00	.00
Left perirenal area	54	26	80	56.3	23.7	80	.09	.22	.31
Right "	60	25	85	59.8	25.2	85	.00	.00	.00
Parietal peritoneum	36	18	54	38.0	16.0	54	.11	.25	.36
Serosa and mesentery	65	25	90	63.4	26.6	90	.04	.10	.14
Omentum	62	31	93	65.5	27.5	93	.19	.55	.74
Diaphragm	43	18	61	43.0	18.0	61	.00	.00	.00
Posterior mediastinum	37	19	56	39.4	16.6	56	.15	.35	.50
Superior "	63	37	100	70.4	29.6	100	.81	1.67	2.48
Neck, anteriorly	32	15	47	33.1	13.9	47	.04	.09	.13
Neck, posteriorly	28	9	37	26.1	10.9	37	.14	.33	.47
Interscapular space	30	8	38	26.8	11.2	38	.38	.91	1.29
Muscles, torso	39	13	52	36.6	15.4	52	.16	.37	.53
Hypoderma, torso	34	9	43	30.3	12.7	43	.45	1.08	1.53
Visceral pleura	67	33	100	70.4	29.6	100	.16	.39	.55
Pericardium	18	6	24	16.9	7.1	24	.07	.07	.24
Left kidney	105	34	139	97.9	41.1	139	.51	1.23	1.74
Right "	100	41	141	99.3	41.7	141	.00	.01	.01
Lungs	67	33	100	70.4	29.6	100	.16	.39	.55
Left suprarenal	47	23	70	49.3	20.7	70	.11	.26	.37
Right "	44	16	60	42.2	17.8	60	.08	.18	.26
Gums	57	13	70	49.3	20.7	70	1.20	2.86	4.06
Mandible	50	12	62	43.7	18.3	62	.91	2.17	3.08
Liver	49	26	75	52.8	22.2	75	.27	.65	.92
Nose and sinuses	38	13	51	35.9	15.1	51	.12	.29	.41
Eyes	34	9	43	30.3	12.7	43	.45	1.08	1.53

Muscles, scapulæ	29	9	38	26.8	11.2	38	.18	.43	.61
Intestines	25	14	39	27.5	11.5	39	.23	.54	.77
Spinal canal	25	9	34	23.9	10.1	34	.05	.12	.17
Metaphysis, tibiæ	25	8	33	23.2	9.8	33	.14	.32	.56
Metaphysis, femora	23	10	33	23.2	9.8	33	.00	.00	.01
Hypophysis	23	10	33	23.2	9.8	33	.00	.00	.01
Muscles, mastication	23	5	28	19.7	8.3	28	.55	1.31	1.86
Skin, torso	22	8	30	21.1	8.9	30	.04	.09	.13
Heart	19	6	25	17.6	7.4	25	.11	.26	.37
Muscles, tongue	13	4	17	12.0	5.0	17	.08	.20	.28
Parathyroids	7	6	13	9.2	3.8	13	.52	1.27	1.79
Thyroid	9	4	13	9.2	3.8	13	.00	.01	.01
Pericranium	8	2	10	7.0	3.0	10	.14	.33	.47
Muscles, anteriorly, thigh	7	3	10	7.0	3.0	10	.00	.00	.00
Spleen	4	10	14	9.9	4.1	14	3.51	8.49	12.00
Stomach	3	5	8	5.6	2.4	8	1.21	2.81	4.02
Meninges	3	2	5	3.5	1.5	5	.07	.17	.24
Brain	1	1	2	1.4	0.6	2	.11	.27	.38
Totals	1868	785	2653	1868.0	785	2653	14.63	33.85	49.49
					2	2			
					2	2			
					n	n			
					—	—			
					1	1			
					0.202	0.202			

perimental errors having a random distribution. To test this hypothesis the values of x^2/m (square of departures divided by expectations) were calculated and presented at the right of the table. Chi-square was 49.49, which with the degrees of freedom equal to 48 was found not to exceed what might have been expected from the foregoing assumptions ($\sqrt{2x^2} - \sqrt{2n-1} = 0.202$). It is even suggestive that the experimental error is unusually small; were it not for the values for the spleen the 2 series could be said to consist of a pattern of 2 highly correlated variables with a minimum of experimental error. The coefficient of correlation on the 2 distributions was +0.922 ($n = 46$, $P = 0.001$ -, significant) and the regression line is linear. The only significant departure from the regression line is in the value for the spleen, in which more metastases were found among treated animals than was to have been expected. It is our belief that the original observations were not correct, and that the error occurred because animals receiving homologous material frequently had massive metastases involving the omentum and often the capsule of the spleen by extension. Such extensions probably should not be considered true metastases to the spleen. When they are eliminated, the data indicate the expected involvement for the spleen. Furthermore, when homologous material was administered to animals which were later inoculated intracutaneously in another series of experiments, no significant preponderance of splenic metastases was noted.

It is therefore concluded that injection of rabbits with an homologous material from the Brown-Pearce tumor does not affect the relative distribution of metastases following transplantation. The constancy and character of the relative distribution of metastases in this tumor is thus confirmed for the third time.^{5, 6}

10366

Distribution of Metastases in the Brown-Pearce Tumor.

IV. Effects of Site of Inoculation.*

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It has been shown that the distribution of metastatic foci in the

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