urine above the level prior to riboflavin injection was in no instance significantly different in the diabetic and non-diabetic groups. It seems apparent that the large urinary volume in the diabetic animals do not "wash out" riboflavin to a significant degree, if at all. Attempts to find a significant correlation between the amount of riboflavin retained and food intake, water intake, gain after dosage with riboflavin, or loss of body weight during the depletion period were unsuccessful.

All diabetic animals including those which received riboflavin developed cataracts after 55 to 65 days while none of the non-diabetic riboflavin-deficient animals developed cataracts during the 110 days of the study.

Conclusion. Studies upon the rate of development of riboflavin deficiency and the response to therapeutic doses of riboflavin, after the development of the deficiency syndrome, failed to show any significant difference in the riboflavin requirement of the alloxan diabetic and the non-diabetic rat. Similarly, riboflavin deficiency was apparently without effect upon the development of cataracts in the diabetic animals.

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Intratesticular Versus Subcutaneous Transplantation of Brown-Pearce Tumor in New Zealand Whites.

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Published data[1] on the susceptibility of various rabbit breeds to the Brown-Pearce tumor failed to include the breed most readily procurable on the open market, namely, the New Zealand White. During the past several years 108 New Zealand white male rabbits have been used as controls or stock animals in experiments with the Brown-Pearce tumor, 42 being inoculated intratesticularly and 66 subcutaneously.

Materials and methods. The 66 animals inoculated subcutaneously belonged to 16 different groups distributed throughout the year as follows: January 24 (1), March 6 (2), March 20 (3), May 22 (5), June 23 (8), June 24 (8), July 24 (9), August 11 (4), September 6 (10), October 9 (8), October 31 (3), November 15 (2), December 13 (1), and December 30 (2).

The 42 animals inoculated intratesticularly belonged to 16 groups distributed as follows: January 24 (1), February 28 (6), June 9 (1), June 23 (1), July 17 (1), August 3 (4), August 30 (2), September 16 (1), September 24 (3), October 16 (2), November 9 (3), November 20 (1), November 22 (3).

The 7 animals comprising the two groups inoculated subcutaneously August 11 and October 31 were 3 months of age, others inoculated subcutaneously and all of those inoculated intratesticularly were animals of 4-12 months of age. Animals inoculated subcutaneously and those inoculated intratesticularly were obtained in almost equal proportions from the same breeders or dealers in Alabama, Louisiana, Illinois, Virginia and Pennsylvania.

As in the earlier studies,[2] a metastatic focus was defined as the presence of one or more tumor nodules at necropsy in any of the

* The work was aided by a grant through the Committee on Growth, of The National Research Council, Acting for The American Cancer Society.

1 Casey, Albert E., *Am. J. Cancer*, 1937, **21**, 446.

2 Casey, Albert E., *Am. J. Cancer*, 1934, **21**, 760; 1936, **26**, 276.

following 50 sites: testis, tunic, cord extension, opposite testicle, cord or tunic, retroperitoneal space, bladder, L. perirenal space, L. kidney, R. perirenal area, R. kidney, R. adrenal, L. adrenal, parietal peritoneum, serosa, omentum and ligaments, intestines, spleen, stomach, liver, pancreas, diaphragm, post-mediastinum, sup. med. and thymus, pleura, lungs, pericardium, heart, ant. cervical region, thyroid, parathyroids, muscle of tongue, muscle of mastication, mandible, teeth (mouth), eyes, pericranium, brain, hypophysis, nose and sinuses, post cerv. region, interscapular space, muscle of scapulae, muscle of thorax and abdo., skin of thorax and abdo., subcut. tissue thorax and abdo., muscle ant. portion of thigh, lower ends of femora, upper ends of tibiae, spinal canal. Paralyzed animals or animals with perforating eye metastases were killed and the day of death listed as the time of occurrence of the paralysis or perforation.

Results. Among the 42 New Zealand white rabbits inoculated intratesticularly there were at the end of a 60-day observation period, 37 with primary tumors which had not regressed (88%), 39 with metastases (93%), and 26 which had died from tumor metastases (mortality 62%). The metastatic foci averaged 13.4 (sites) per animal with metastases, 12.3 (sites) per animal with tumor at necropsy, and 11.4 (sites) per animal inoculated (Var. mean = 2.1). The primary tumors averaged by water displacement at necropsy 16.2 cc per animal with primary tumor, 16.2 cc per animal with tumor, and 15.0 cc per animal inoculated. The metastatic tumor averaged 105.6 cc by water displacement at necropsy per animal with metastases, 97.4 cc per animal with tumor and 90.5 cc per animal inoculated.

Among the 66 New Zealand white rabbits inoculated subcutaneously there were at the end of the 60-day observation period 31 with primary tumor which had not regressed (47%), 33 with metastases (50%), and 9 which had died from the effects of tumor metastases (mortality 13%). The metastatic foci averaged 8.2 (sites) per animal with metastases, 6.4 (sites) per animal with tumor, and 3.2 (sites) per animal inoculated (Var. mean = 0.59). The primary tumors averaged by water displacement at necropsy 48.6 cc per animal with primary tumor, 45.7 cc per animal with tumor at necropsy, and 22.8 cc per animal inoculated. The metastatic tumor averaged by water displacement at necropsy 33.2 cc per animal with metastases, 26.1 cc per animal with tumor, and 13.1 cc per animal inoculated.

Each of the above listed differences between the course of tumor after transplantation by the subcutaneous and by the intratesticular routes was statistically significant.[3] For instance, the difference in the mortality of 13% and 62% was significant ($X^2 = 25.1$, $N = 1$, $P = 0.0001$—), and in the number of metastatic foci per animal inoculated of 11.4 and 3.2 (diff. $= 8.2 \pm 1.64$; $t = 5.0$; $P = 0.0001$—).

Generally, the two best criteria for this neoplasm are the mortality from the tumor in

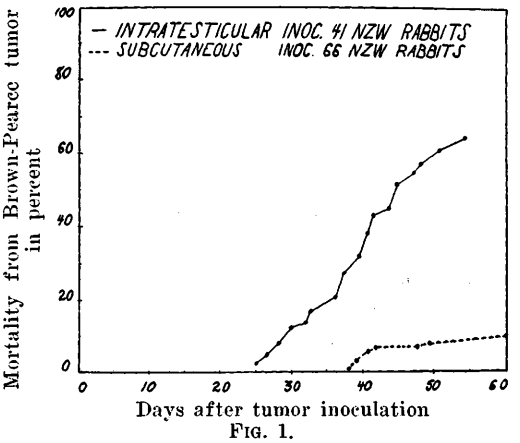

FIG. 1.

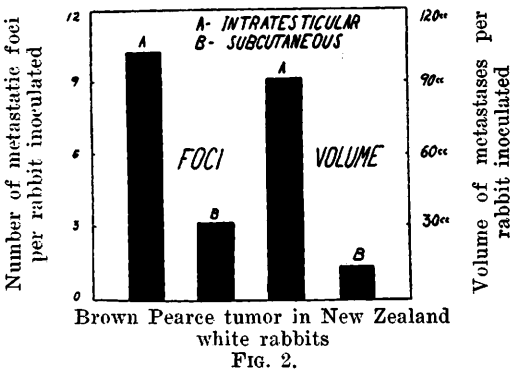

FIG. 2.

[3] Fisher, R. A., Statistical Methods for Research Workers, 1938, 7 ed., Oliver and Boyd, London.

days after inoculation, and the number of metastatic foci determined by careful necropsy and confirmed by microscopic sections.[2] These values are shown in Fig. 1 and 2.

Discussion. The total primary and metastatic tumor per animal with metastases was 82 cc per animal inoculated subcutaneously, and 122 cc per animal inoculated intratesticularly; or an average per metastatic focus of 8.9 cc (for the 9.2 primary and metastatic foci) in the former, and 8.4 cc (for the 14.4 primary and metastatic foci) in the latter. It is obvious that the average rate of growth of the tumor was approximately the same in any given site. Under the skin the primary tumor grew larger as there was abundant area to expand. In the testis the growth was confined and spread up the cords to the perirenal areas, retroperitoneal space and peritoneal cavity by simple extension. It has been proven that the pattern of local spread is determined by the site of injection of this tumor;[4] also the pattern of the distant metastases in the Brown-Pearce and in other tumors is not altered or affected by the site origin of the primary tumor[4] nor by the breed of rabbit employed.[5]

The differences between the subcutaneous and the intratesticular routes were not due to the season of the year in which the animals were inoculated.[6] The rabbits inoculated during October, November, December, January, February and March averaged 12.2 metastatic foci among the 16 rabbits transplanted intratesticularly and 4.68 foci among the 22 transplanted subcutaneously; the rabbits inoculated during May, June, July, August and September averaged 10.9 metastatic foci for the 21 rabbits transplanted intratesticularly and 2.48 metastatic foci for the 44 rabbits transplanted subcutaneously. Nor were the differences due to age since the elimination of the 7 animals 3 months of age did not appreciably affect the mean number of metastatic foci or the mortality.

The possibility was considered that the New Zealand Whites obtained from such widely separated areas as Pennsylvania, Illinois, and Alabama might represent stock variations. Such stock variations undoubtedly did occur but the animals injected by the two routes were obtained in roughly proportioned numbers from the various areas. Furthermore, Havana, Himalayana, and Flemish stock obtained also from dealers in the various states have given almost the same data on mortality and metastatic foci as was obtained from inbred lines some 15 years ago.[1] It still remains true that no Havana rabbit has died from the Brown-Pearce tumor, although metastases have occasionally been noted. Approximately 30% of all animals (including all breeds and hybrids) inoculated subcutaneously have died from metastases within a 2-3 months observation period. This may explain a variety of results reported by various authors trying to produce immunity by the subcutaneous route. Nozu was the first to observe metastases of the Brown-Pearce tumor by intracutaneous inoculation alone[7] and Casey induced metastases by the intracutaneous route using the Brown-Pearce XYZ factor.[8]

Brown and Pearce noted the marked tendency to metastasis following intratesticular inoculation of the rabbit tumor and an absence of metastasis following intra- and subcutaneous inoculation.[9] Pearce and Brown were the first to point out that rabbits inoculated with the Brown-Pearce tumor intra- or subcutaneously or intramuscularly "developed an immunity which was sufficient to protect them from subsequent inoculation by the same route or from inoculation made into the

[4] Casey, Albert E., and Pearson, Bjarne, PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 234.

[5] Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 223.

[6] Brown, Wade H., Pearce, Louise, and Van Allen, C. M., PROC. SOC. EXP. BIOL. AND MED., 1924, **21**, 371; Pearce, Louise, and Van Allen, C. M., PROC. SOC. EXP. BIOL. AND MED., 1925, **22**, 448; *J. Exp. Med.*, 1927, **45**, 483; Pearce, Louise, and Brown, Wade H., *J. Exp. Med.*, 1927, **45**, 727.

[7] Nozu, Y., *Acta dermat.*, 1933, **26**, 66.

[8] Casey, Albert E., *Am. J. Cancer*, 1934, **21**, 776.

[9] Brown, Wade H., and Pearce, Louise, *J. Exp. Med.*, 1923, **37**, 799.

testicles."[10] Their observations were confirmed by Casey[8] and by Besredka, Mazat, Besnard, Gross, Bardach, Laval,[11] Saphir, Appel, Strauss,[12] Cheever, and Janeway.[13] Besredka and Gross reported that this immunity could be produced only by the tumor cells and that it could not be induced by the intracutaneous inoculation of normal tissues.[11] In Fig. 1 it will be seen that the New Zealand white rabbit shows no evidence of developing at about 40 days an immunity or resistance following intratesticular inoculation as is true for this same breed following subcutaneous inoculation or for common hybrids by intratesticular inoculation as reported by Brown and Pearce[9,10] and by Malluche.[14]

Summary and conclusions. Standard values were compiled for the Brown-Pearce tumor in New Zealand white male rabbits, 42 being inoculated intratesticularly and 66 subcutaneously. The breed was highly resistant to subcutaneous inoculation, (mortality, 13.6%; metastatic foci, 3.2; metastatic tumor 13.1 cc per animal inoculated) and susceptible to intratesticular inoculation (mortality 62.7%, metastatic foci 11.4 and metastatic tumor 90.5 cc per animal inoculated). Between 40 and 60 days after intratesticular inoculation the mortality curve continued to rise at about the same rate as between 20 and 40 days. This was in contrast to the immune or resistance reaction which seemed to set in between 40 and 60 days following subcutaneous inoculation of this breed, or following intratesticular inoculation of most other breeds.

10 Pearce, Louise, and Brown, Wade H., *J. Exp. Med.*, 1923, **37**, 811.

11 Besredka, A., Mazat, L., and Besnard, P., *Comp. rend. Acad. d. sc.*, 1935, **201**, 170; Gross, L., *Am. J. Cancer*, 1937, **31**, 609; Besredka, A., and Bardack, M., *Comp. rend. Acad. d. sc.*, 1936, **203**, 2193; Besredka, A., *Cancer Bruxelles*, 1935, **12**, 115; Besredka, A., Mazat, L., Laval, P., and Besnard, P., *Ann. Inst. Pasteur*, 1936, **36**, 125; Besredka, A., and Gross, L., *Ann. Inst. Pasteur*, 1936, **57**, 342; 1938, **60**, 5, 465; 1939, **62**, 253.

12 Saphir, O., and Appel, M., *Am. J. Cancer*, 1940, **38**, 55; Saphir, O., Appel, M., and Strauss, A. A., *Cancer Research*, 1941, **1**, 545.

13 Cheever, F. S., and Janeway, C. A., *Cancer Research*, 1941, **1**, 23.

14 Malluche, H., *Beitr. Z. klin. Chir.*, 1938, **167**, 481.

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Selective Blocking of Host Resistance to Malignant Neoplasm (Brown-Pearce Tumor in New Zealand White Rabbits).

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In the preceding paper[1] it was reported that the New Zealand White rabbit is highly susceptible following intratesticular and highly resistant following subcutaneous transplantation of the Brown-Pearce tumor. In the mortality curve an immune or resistance reaction could be detected at about 40-60 days following subcutaneous inoculation but little or none following intratesticular inoculation of the tumor. The present experiments were designed to test the extent to which the XYZ factor[2] might overcome this immune or resistance reaction of the New Zealand white to this tumor following subcutaneous inoculation.

* The work was aided by a grant from the Committee on Growth of the National Research Council, acting for the American Cancer Society.

1 Casey, Albert E., Meyers, L., and Drysdale, George R., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 576.

2 Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 816; *Am. J. Cancer*, 1934, **21**, 760; 1936, **26**, 276; 1939, **35**, 354.