pathways develop from roots which normally contribute nothing to the forepad.

This may offer additional explanation for early relapse following preganglionic section for Raynaud's disease in the hands, and for the fact that operations for this disease are generally more successful in the feet than in the hands.

13802

Quantitative Studies on Tumor Production in Mice by Benzpyrene.

RAYMOND G. GOTTSCHALK.* (Introduced by J. Furth.)

From the Department of Pathology, University of Liége, Belgium.†

Quantitative studies on the response of animals to benzpyrene do not extend to the smallest effective doses; precise data on the effect of small amounts of other carcinogens are likewise scarce.

Brock, Duckrey and Hamperl[1] inserted a collodion sac containing crystals of 3-4 benzpyrene (500 to 25,000 γ) into the peritoneum of rats. They estimated that about 1 γ of benzpyrene passed the collodion membrane daily; this was sufficient to induce sarcoma in the majority of animals. Oberling and M. and P. Guérin[2] obtained sarcomata in 4 of 35 rats that had received a single subcutaneous injection of 100 γ of benzpyrene in olive oil and in 1 of 10 rats injected with 50 γ of benzpyrene. Shimkin and Andervont[3] injected male mice of C_3H stock with 0.2 cc of tricaprylin containing from 250 to 2000 γ of benzpyrene. All animals developed tumors after injection of 2000 and 1000 γ; 19 of 20 and 18 of 20, respectively, were positive after injection of 500 γ and 250 γ. The same amounts of benzpyrene were injected into hybrid mice, but the results were more irregular, and tumors failed to appear in at least one-third of the injected animals. Bryan and Shimkin,[4] analyzing the values of latent period of tumors obtained by the former authors in C_3H mice (87 to

* Graduate Fellow of the Belgian American Educational Foundation.

† These experiments were performed in the Department of Pathology of the University of Liége from November, 1938, to May, 1940.

1 Brock, N., Duckrey, H., and Hamperl, H., *Arch. f. exp. Path. u. Pharmakol.*, 1938, **189**, 709; *Arch. f. klin. Chirurg.*, 1938, **194**, 250.

2 Oberling, Ch., Guérin, M. and P., *Bull. Assn. Fr. Etude Canc.*, 1939, **28**, 198.

3 Shimkin, M. B., and Andervont, H. B., *J. Nat. Canc. Inst.*, 1940, **1**, 57.

4 Bryan, W. R., and Shimkin, M. B., *J. Nat. Canc. Inst.*, 1941, **1**, 807.

108 days), found a straight line relationship between average latent period and the logarithm of the injected dose, excluding the 2000 γ benzpyrene values, as no further enhancement of activity was obtained above 1000 γ. This relationship was confirmed by reliable data published by other investigators[5, 6] on the latent period of tumors induced by 1-2-5-6 dibenzanthracene. Smaller amounts of this carcinogen have also been tested. Two of 167 mice injected by Dobrovolskaia-Zavadskaia[5] with 2.5 γ of dibenzanthracene developed sarcomata; whereas 1.25 γ failed to induce tumors in 158 mice. Lettinga[6] obtained tumors in 4 of 20 mice after subcutaneous injection of 12.5 γ of dibenzanthracene (divided into 5 injections), but failed in 20 mice after injection of 5 γ. Shear[7, 8] produced a sarcoma in 1 of 18 strain A mice 13 months after subcutaneous insertion of a 0.001% dibenzanthracene-cholesterol pellet containing 0.4 γ of the carcinogen, but failed in 15 and 18 mice with 0.1% and 0.01% pellets. The minimum concentrations of the carcinogen in the cholesterol pellets with which Shear and Ilfeld[9] produced sarcomata in strain D mice were 1% of dibenzanthracene and of methylcholanthrene (70 to 600 γ) and 5% of benzpyrene (350 to 3000 γ). Oberling, Sannié and P. and M. Guérin[10] obtained local sarcomata in rats after intracerebral application of 1 drop of 1:1000 methylcholanthrene solution in oil.

Experimental. The mice used were of an albino stock kept at the University of Liége. Eight groups of 20 mice received a single injection under the skin of the abdomen of 3-4 benzpyrene‡ dissolved in 0.5 cc of neutral olive oil. The dose per mouse in each group varied by geometric progression, as shown in Table I, from 4×10^3 to 4×10^{-2} γ. The oil alone produced no tumors on repeated injection into 15 mice.

Table I indicates that 4 γ are the smallest amount of benzpyrene which produced a tumor when administered in a single dose subcutaneously in 0.5 cc of olive oil. Four-tenths γ and .04 γ failed to produce sarcomata at the site of injection. The two carcinomata of the breast observed were probably spontaneous. The percent of tumors obtained was larger when larger amounts of the chemical

5 Dobrovolskaia-Zavadskaia, N., *C. R. Soc. Biol.*, 1938, **129**, 1055.

6 Lettinga, T. W., *De Carcinogene Werking van kleine Doses 1-2-5-6 Dibenzanthraceen*, Academisch Proefschrift, Amsterdam; Van Gorcum and Co., Assen, 1937.

7 Shear, M. J., *Am. J. Canc.*, 1936, **26**, 322.

8 Shear, M. J., and Lorenz, E., *Am. J. Canc.*, 1939, **36**, 201.

9 Shear, M. J., and Ilfeld, F. W., *Am. J. Path.*, 1940, **16**, 287.

10 Oberling, Ch., Sannié, Ch., Guérin, P. and M., *C. R. Soc. Biol.*, 1939, **131**, 455.

‡ Obtained from Meurice, Union Chimique Belge.

TABLE I.
Results of Subcutaneous Injection of Diminishing Amounts of Benzpyrene.

Amt in γ	No. of mice*	No. of tumors†	% tumors	Avg latent period (days)	Avg survival tumor-bearing mice (days)‡
4000	9	6	66.6	112	130
1265	10	7	70	122	154
400	5	1	20	145	178
126.5	12	8	66.6	155	199
40	5	1	20	122	215
4	9	1	11.1	187	260
0.4	15	0 (2§)		(226)	(255)
0.04	9	0 (1§)		(245)	(294)

*Alive at the appearance of the first tumor. A large number died early because of fighting; some were killed for histological examination.

†Sarcomata, occasionally with epithelioma.

‡The mice were allowed to die. Ten mice were killed when bearing large tumors. Three had been injected with 4000 γ, 3 with 1265 γ, 1 with 126.5 γ, 1 with 40 γ, 1 with 4 γ and 1 with 0.04 γ. In calculating the average length of survival, the day of death of these animals was considered to be the tenth day after they had been killed.

§Adenocarcinoma of breast.

were injected. A discordant value (400 γ group) can be explained by the small number of animals used; moreover, the mice were not of an inbred stock. On the whole, these data confirm and extend those of Shimkin and Andervont.[3]

There is a definite trend toward lengthening of the latent period with the decrease of the amount injected. The values obtained between 126.5 and 4000 γ fit a linear relation between the average latent period and the logarithm of injected dose, and the slope is similar to that derived from the values of Shimkin and Andervont.[3, 4] Whether this relation applies also to doses of benzpyrene below 126.5 γ cannot be stated because of the small number of tumors produced.

The tumors were regularly preceded by induration around the oil cyst, but carcinogenesis did not always follow the induration. Microscopically, areas of fibrosis and chronic inflammation surrounded the oil cyst. In a few cases small groups of seemingly malignant cells were seen about the oil cyst; these animals were reported as negative, as the onset of a tumor was dated arbitrarily from the appearance of a nodule measuring approximately 1 mm across.

The average survival time of tumor-bearing mice is shortened in a linear relation with the logarithm of the injected dose. Shimkin[11] has already shown that the mortality curve parallels the curve of appearance of tumors, despite the unpredictable rate of tumor growth.

In order to study the *rôle of volume* in carcinogenesis, a constant

[11] Shimkin, M. B., *J. Nat. Canc. Inst.*, 1941, **1**, 761.

TABLE II.
Results of Injection of 400 γ of Benzpyrene in Varying Amounts of Oil.

Vol. oil (cc)	No. inj. mice	No. of tumors*	Avg latent period (days)
1	20	8	154
0.5	20	10	107
0.25	20	9	103
0.125	20	11	144
0.0625	20	4	128
0.03	20	5	133
0.015†	20	2	150
Total	140	49	128

*Sarcomata—Results read on the 204th day.
†Soluble at 40°C.

amount of benzpyrene (400 γ) was dissolved in olive oil varying in amount from .015 cc to 1 cc and injected in groups of 20 mice each.

When the solvent was .125 cc or more, at least 40% of tumors were produced. When the solvent was .0625 cc or less, the percent of tumors produced was below 25. The corresponding average latent periods were, however, without relation to the volume injected. These data may perhaps be explained by assuming that if a concentrated solution is injected, a relatively smaller number of cells is exposed to the irritant, or that the carcinogen is more quickly eliminated, or that the tissue derangement, which is believed to favor initiation of tumor growth, is less.[12]

The *relationship between the injected oil and tumor* or inflammatory reaction was studied systematically. The autopsies were performed under ultraviolet light, using a mercury lamp with Wood's filter. After shaving the skin, the presence of a very strong fluorescence often enabled the localization of an oil cyst beneath the skin, and minute amounts of fluorescent material could be identified in the tissues. This procedure guided the selection of areas for microscopic study. In mice injected with 4 γ or less fluorescence was not sufficient for the localization of the carcinogen.

In order to study the rate of absorption of the carcinogenic oil, the oil cysts were punctured with a thin needle and washed from 3 to 5 times with spectrographically pure petroleum ether until the fluorescence disappeared, but traces of fluorescent product could sometimes not be washed out of the wall of the cyst. The oil and petroleum ether mixture was evaporated in a vacuum and in the dark to a constant weight. The samples were kept in the ice box and in the dark to avoid disappearance of the fluorescence and of the ab-

[12] Dunning, W. F., Curtis, M. R., and Wood, F. C., *Am. J. Canc.*, 1940, **39**, 70.

sorption spectrum characteristic of benzpyrene. The uninjected solutions of benzpyrene in oil were not as labile. The material dissolved in petroleum ether was tested by absorption-spectrography under variable thickness (25 cc cups). The technic used allowed a rapid determination of amounts of 3-4 benzpyrene above 1 γ; the precision was about $\pm 5\%$. The amount of 3-4 benzpyrene identified was lower than expected from the weight of the remaining oil. The results also show that the rate of resorption of the oil was irregular and without relation to the amount of benzpyrene injected or to the appearance of a tumor. These experiments will be more fully described when the data are obtainable from Belgium.

Thus, despite the variability of local factors, such as the encapsulation and the rate of resorption of the carcinogenic oil, despite the irregular rate of growth of the neoplastic elements, definite relationships can be found between the amount of carcinogen used and the number of induced tumors, their latent period, or the average survival of tumor-bearing mice.

Summary. Amounts of benzpyrene ranging between .04 γ and 4 mg dissolved in 0.5 cc of olive oil were injected subcutaneously into mice. The smallest amount producing a sarcoma was 4 γ. The data suggest a linear relationship between the logarithm of the injected dose and the average latent period of tumors, as found by Bryan and Shimkin. A similar relationship was found between dose and average length of survival of the tumor-bearing animals. When dissolved in .125 to 1 cc of oil, 400 γ of benzpyrene produced tumors in 40% or more of the mice. When dissolved in from .0625 to .015 cc of oil, the incidence of tumor production was 25% or less. No relationship was found between average latent period and volume of injection. A technic is described for the study of the rate of resorption of benzpyrene and oil.

I am indebted to Dr. Jean Firket, Department of Pathology, University of Liége, for his kind advice; to the late Mr. Victor Henri and to Mr. Michel Herquet, Department of Physical Chemistry, University of Liége, for their coöperation in the spectrographical analyses.