strated by the survival of 92% of 630 mice injected intraäbdominally with the sulfanilamide-toxin mixture and the death of all 195 controls which received toxin only. The *in vivo* action of sulfanilamide, administered either before or subsequent to the toxin, was less marked.

The toxin of *Clostridium welchii* was likewise inactivated *in vitro* by sulfanilamide. Eighty-four per cent of 120 and 86% of 140 mice, injected intraäbdominally and intramuscularly, respectively, with the toxin-sulfanilamide mixture survived. *In vivo,* sulfanilamide protected 83% of 120 mice, when administered after the intraäbdominal injection of the toxin, and 90% of 120 mice after intramuscular inoculation. Only 8% of 60 control mice, injected intraäbdominally, and 12% of 60 control mice, injected intramuscularly, lived.

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## Effect of Petroleum Ether Extract of Mouse Carcasses as Solvent in Production of Sarcoma.*

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The production of sarcomata in mice may be influenced by the solvent used for the carcinogenic hydrocarbon.[1] Most experiments have been carried out with vegetable oils, lard, cholesterol or paraffin which are effective vehicles for 1:2:5:6-dibenzanthracene, 3:4-benzpyrene and methylcholanthrene. The increase in the incidence of skin tumors in mice following the application of mouse fat to the skin before tarring, as reported by Watson and Mellanby,[2] led us to investigate the effect of a petroleum ether extract of mouse tissues as a solvent for 3:4-benzpyrene in the production of connective tissue sarcoma.

The extract was prepared by refluxing fresh minced mouse carcasses, from which the stomach and intestines had been excised, with petroleum ether (maximum boiling point 50°C) for 16 hours. The petroleum ether was removed by distillation under reduced pressure.

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1 Peacock, P. R., and Beck, S., *Brit. J. Exp. Path.*, 1938, **19**, 315.

2 Watson, A. F., and Mellanby, E., *Brit. J. Exp. Path.*, 1930, **11**, 311.

The resulting mixture was turbid, yellow and oily at 37°C. A gray-white, greasy, amorphous precipitate formed on cooling. The whole extract consisted largely of neutral fats and free fatty acids.

Sesame oil and colloidal solutions of 3:4-benzpyrene were also used. The colloidal material was prepared by the gelatin method of Boyland.[3] Each injection, representing 0.25 mg benzpyrene, was made in the subcutaneous tissues.

Three groups of 50 C57 black mice received a single injection of 3:4-benzpyrene in the inguinal region dissolved in sesame oil, petroleum ether extract of mouse carcasses or as a colloidal solution. In a fourth group of 50 mice, each animal received a single injection of each of the 3 solutions. All of the mice were obtained from the Roscoe B. Jackson Memorial Laboratory and were 5 to 6 weeks old when the injections were made. They were observed once weekly. The time when a progressively growing mass was first noted was taken as the appearance time of the tumor. Surviving animals were killed 32 weeks after injection. All diagnoses were confirmed by histologic examination. The effective total method was applied to the results.[4]

One tumor appeared among the 44 mice that received only a single injection of benzpyrene in the petroleum ether extract of mouse carcasses (Table I). The mice that were given benzpyrene in sesame oil developed more tumors than did those receiving colloidal benzpyrene. In the group of mice that was injected with benzpyrene in each of the 3 solutions only one tumor appeared when the solvent was petroleum ether extract of mouse carcasses (Tables II and III). It is possible that the much higher incidence of tumors resulting from the sesame oil and colloidal solutions caused the death of the animals before they had the opportunity to develop sarcoma at the site of injection of the extract. The experience with the group that received benzpyrene in petroleum ether extract of mouse carcasses alone makes this explanation less probable.

Andervont[5] reported a difference in sarcoma production of male and female C57 black mice injected with 1:2:5:6-dibenzanthracene and methylcholanthrene. This was most pronounced in the latent interval of tumor production. In our series the incidence of tumors in the sexes was not significantly altered when sesame oil was the solvent. More male mice developed sarcomata when colloidal benzpyrene was administered. The latent interval to production of sarcoma was approximately the same for males and females except

[3] Boyland, E., *Lancet*, 1932, **2**, 1108.

[4] Fieser, L. F., *Am. J. Cancer*, 1938, **34**, 37.

[5] Andervont, H. B., *Pub. Health Rep.*, 1938, **53**, 1647.

TABLE I.

Sarcomata in C57 Black Mice Receiving a Single Injection of 0.25 mg 3:4-Benzpyrene in the Inguinal Region.

| Weeks No. of mice | Character of injection | Sex | No. | | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 | 28 | 30 | Total No. tumors | | Died or killed without tumors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | a. No. of new tumors | | | | | | | | | | | | | | |
| | | | | b. Accumulated % mice with tumors | | | | | | | | | | | | | | |
| 46 | Dissolved in .25 cc sesame oil | M | 21 | a | 1 | 2 | 1 | 3 | 5 | 3 | | | 1 | 1 | | 17 | | |
| | | | | b | 5 | 14 | 19 | 33 | 57 | 71 | | | 76 | 81 | | | 36 | |
| | | | | | | | | | | | | | | | | | | 10 |
| | | F | 25 | a | | 2 | | 10 | 3 | | | 3 | | 1 | | 19 | 78% | |
| | | | | b | | 8 | | 48 | 60 | | | 72 | | 76 | | | | |
| 46 | Colloidal solution 0.5 cc | M | 20 | a | | 1 | | 3 | 3 | 3 | 1 | | 1 | | | 12 | | |
| | | | | b | | 5 | | 20 | 35 | 50 | 55 | | 60 | | | | 23 | |
| | | | | | | | | | | | | | | | | | | 23 |
| | | F | 26 | a | | | | 1 | 2 | 1 | 2 | 2 | 2 | 1 | | 11 | 50% | |
| | | | | b | | | | 4 | 11 | 15 | 23 | 30 | 38 | 42 | | | | |
| 44 | Dissolved in .25 cc extract | M | 17 | a | | | | | | | | | | | | 0 | | |
| | | | | b | | | | | | | | | | | | | 1 | |
| | | | | | | | | | | | | | | | | | | 43 |
| | | F | 27 | a | | | | | | | 1 | | | | | 1 | 2% | |
| | | | | b | | | | | | | 4 | | | | | | | |

TABLE II.
Sarcomata in C57 Black Mice Receiving 3 Separate Injections of 0.25 mg 3:4-Benzpyrene.

| Time in weeks<br>Character of injection | Sex | No. | | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 | 28 | 30 | Total No. tumors | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | a | No. of new tumors | | | | | | | | | | | | |
| | | | b | Accumulated % of mice with tumors | | | | | | | | | | | | |
| Dissolved | M | 19 | a | | | 3 | 3 | 4 | 1 | | | | 1 | | 12 | |
| in .25 cc | | | b | | | 16 | 32 | 53 | 58 | | | | 63 | | | 26 |
| sesame oil | F | 26 | a | 2 | 2 | 4 | 2 | 2 | 2 | | | | | | 14 | 57% |
| | | | b | 8 | 15 | 30 | 38 | 46 | 53 | | | | | | | |
| Colloidal | M | 19 | a | | | 3 | 2 | 3 | 1 | 2 | 1 | | | | 12 | |
| solution | | | b | | | 16 | 25 | 42 | 47 | 58 | 53 | | | | | 19 |
| 5 cc | F | 26 | a | 1 | 2 | | 1 | 2 | | | | 1 | | | 7 | 41% |
| | | | b | 4 | 11 | | 15 | 23 | | | | 27 | | | | |
| Dissolved | M | 19 | a | | | | | | | | | | | | 0 | |
| in .25 cc | | | b | | | | | | | | | | | | | 1 |
| extract | F | 26 | a | | | | | | | | 1 | | | | 1 | 2% |
| | | | b | | | | | | | | 4 | | | | | |

TABLE III.
Sex Distribution of Sarcomata in C57 Black Mice Receiving 3 Separate Injections of 0.25 mg 3:4-Benzpyrene.
(Effective total—45; 19 male, 26 female.)

| Mice | Male | Female | Total |
|---|---|---|---|
| Died without tumor | 1 | 8 | 9 |
| One tumor only | 12 | 14 | 26 |
| Sesame oil | 6 | 10 | 16 |
| Colloid | 6 | 3 | 9 |
| Extract | 0 | 1 | 1 |
| Two tumors | 6 | 4 | 10 |

in the group receiving a single injection of colloidal benzpyrene. It is questionable whether the greatest difference, 3.1 weeks, is statistically significant.

Factors involved in the extreme reduction in the incidence of sarcoma following the injection of 3:4-benzpyrene in petroleum ether extract of mouse carcasses are being investigated.