M. L., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 621.

6. Patterson, E. L., Milstrey, R., Stokstad, E. L. R., *ibid.*, 1957, v95, 617.

7. Nesheim, M. C., Scott, M. L., *J. Nutrition*, 1958, v65, 601.

8. Proctor, J. F., Hogue, D. E., Warner, R. G., *J. Animal Sci.*, 1958, v17, 1183.

9. Muth, O. H., Oldfield, J. E., Remmert, L. F., Schubert, J. R., *Science*, 1958, v128, 1090.

10. Drake, C., Grant, A. B., Hartley, W. J., *New Zealand Vet. J.*, 1960, v8, 4.

11. Jolly, R. D., *ibid.*, 1960, v8, 11.

12. ———, *ibid.*, 1960, v8, 13.

13. Dam, H., Søndergaard, E., *Experientia*, 1957, v13, 494.

14. Maplesden, D. C., Loosli, J. K., *J. Dairy Sci.*, 1960, v43, 645.

Received October 23, 1961. P.S.E.B.M., 1962, v109.

Effect of a Single Application of Croton Oil in Skin Carcinogenesis.* (27089)

LORENZO TOMATIS, BENEDETTO TERRACINI AND PHILIPPE SHUBIK
Division of Oncology, The Chicago Medical School, Chicago, Ill.

It was previously demonstrated that when various dosages of 9,10-dimethyl-1,2-benzanthracene dissolved in acetone were applied to the skin of Swiss mice as single applications, it required 200 μg for effective carcinogenic action. Doses of 20 and 50 μg were without effect and a dose of 100 μg gave rise to but a few benign tumors. A dose of 200 μg gave rise to a high incidence of tumors, both benign and malignant. Above a dose of 200 μg, no additional response was noted(1,2). One reason that was suggested for this dose response behavior is that the injury inflicted by the doses of 200 μg and over of this necrotizing carcinogen plays a role. Injury has been shown by several investigators to play a secondary role in carcinogenesis in the skin(3,4,5,6) and analysis of such experiments has provided the experimental basis for considering this process as a 2-stage phenomenon(7,8,9). It might therefore be thought that at the 200 μg level an additional factor associated with increased injury comes into play and that this provides a promoting stimulus enabling the carcinogen to manifest its full activity. It appeared in our original study that injury might play such a role; however, injury to the skin is extremely difficult to qualify and the current experiment was designed to test the hypothesis. Croton oil has been studied in many experiments as a promoting agent in skin carcinogenesis(2,1,11); it is well known to have an injurious effect on many tissues although the mechanism of action in promotion is still obscure. In the previous studies with croton oil, the agent has always been applied repeatedly. In the present investigation, groups of mice were given single applications of either 100 or 200 μg of DMBA alone or combined with croton oil.

Materials and methods. The mice used were Swiss females, 7 to 9 weeks old at the beginning of experiment, originating from mice obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine and bred in this department since 1951. The mice were housed in plastic cages on wood shavings, fed Rockland mouse diet and given water *ad libitum*. The carcinogen 9,10-dimethyl-1,2-benzanthracene (DMBA) was obtained from Eastman Organic Chemicals, then purified by chromatography on fluorisil in this laboratory. Croton oil was obtained from Boots Ltd., England.

Both the croton oil and the DMBA were applied dissolved in fluorescence free acetone, using a calibrated micropipette. The solutions were applied to the interscapular region of the mice using an area approximately 1.5 per 1.5 cm shaved free of hair.

Mice were checked each week and all the

* This investigation was supported by grant from Nat. Cancer Inst., Nat. Inst. Health, U.S.P.H.S.

TABLE I. Distribution Pattern of Induced Tumors.

Treatment	No. of mice	Total TBA*	Total epidermal tumors	Avg latent period (wk)	Total regressing tumors	Carcinomas	Sarcomas
1) 200 μg DMBA in acetone, once	30 ♀	12	19	17.8	6	6	1
2) 200 μg DMBA in a 0.2% sol. of croton oil in acetone, once	30 ♀	14	22	18.1	11	2	1
3) 200 μg DMBA in a 2% sol. of croton oil in acetone, once	30 ♀	8	13	19.0	7	3	1
4) 100 μg DMBA in acetone, once	30 ♀	5	8	15.5	4	0	0
5) 100 μg DMBA in a 0.2% sol. of croton oil in acetone, once	20 ♀	3	3	—	1	1	0
6) 100 μg DMBA in a 2% sol. of croton oil in acetone, once	30 ♀	2	2	—	0	—	1

* Tumor bearing animals.

lesions were graphically recorded. All the tumors present for over 1 week were considered. The animals were kept until death or were killed if in poor condition. The survivors were sacrificed after the 75th week following the application. All mice were completely autopsied, with the exception of 4 lost through cannibalism and complete histological study was undertaken.

Experimental and results. *Six* groups of mice were used. Each consisted of 30 mice with the exception of the 5th group in which only 20 mice were used.

Group 1 were given a single dose of 200 μg of DMBA dissolved in 50 μl acetone. *Group 2* were given a single dose of 200 μg of DMBA dissolved in 50 μl of a 0.2% solution of croton oil in acetone. *Group 3* were given a single dose of 200 μg of DMBA dissolved in 50 μl of a 2.0% solution of croton oil in acetone. *Group 4* were given a single dose of 100 μg of DMBA dissolved in 50 μl of acetone. *Group 5* were given a single dose of 100 μg DMBA dissolved in 50 μl of a 0.25% solution of croton oil in acetone. *Group 6* were given a single dose of 100 μg of DMBA dissolved in 50 μl of a 2.0% solution of croton oil in acetone. The results are summarized in Table I.

It can be seen that previous findings with single applications of DMBA in acetone were confirmed. Whereas a single dose of 200 μg in acetone gave rise to a total of 19 tumors in 12 of 30 treated mice, 7 of which were malignant, the dose of 100 μg gave rise to only 8 tumors in 5 of the 30 treated mice; of these 4 regressed and none were malignant.

When croton oil was added in the 2 concentrations used, no augmentation of effect was noted in any instance. In the case of a dose of 200 μg applied in the lower concentration of croton oil (0.2%) a similar number of tumors was observed, although less became malignant. In the experiment using 200 μg of DMBA in the higher concentration of croton oil (2.0%) a decreased tumor incidence was observed. In the instance of the experiments using 100 μg of DMBA dissolved in the 2 concentrations of croton oil the minimal effect of the 100 μg of DMBA was almost abolished.

The doses of croton oil used in the study did not give rise to ulceration of the skin although the higher concentration did give rise to obvious reddening and thickening of the skin over and above the effect induced by the carcinogen.

Conclusions. In our prior studies we had established that a single dose of 200 μg of the carcinogen DMBA is highly effective, giving rise to tumors of the skin in almost 50% of treated Swiss mice, of which a high proportion appear malignant, namely 28% become squamous cell carcinomas(2). A

dose of 100 μg of DMBA, on the other hand, is a borderline carcinogen giving rise to a small proportion of papillomas many of which regress and few of which progress to malignancy. This result was confirmed. From other studies it is well known that a dose of 100 μg is an effective initiating dose of this carcinogen for the skin; that is, if it was followed by repeated applications of croton oil or some other promoting stimulus, many tumors would appear(10,11). In the present study this dose of carcinogen was combined with a single application of croton oil at 2 dosages. In no instance did croton oil, thus administered, give rise to any promoting effect, but to the contrary appeared, if anything, to engender a reduction in tumor incidence. This finding applied not only to combinations with the 100 μg dose of DMBA, but also to the 200 μg dose.

It thus seems clear that the difference in the carcinogenic capabilities of doses of 100 μg or 200 μg of DMBA cannot be explained on the basis of additional "injury" inflicted by the higher dose, if the "injury" is of the same type as that caused by croton oil. It is clear that the promoting effects of croton oil are quite distinct from the initiating and carcinogenic actions of DMBA since croton oil requires repeated applications to manifest its effect and has, if anything, the reverse effect under the conditions of the present expriment. The qualitative nature of the tumors seen in the croton oil treated groups appears consistent with that observed in many previous studies in that there was a predominance of benign and regressing lesions(2).

1. Terracini, B., Shubik, P., Della Porta, G., *Cancer Res.*, 1960, v20, 1538.
2. Shubik, P., *Acta Unio Int. Contra Cancrum*, 1961, v17, 21.
3. Pullinger, B. D., *J. Path. Bact.*, 1945, v57, 467.
4. Gwynn, R. H., Salaman, M. H., *Brit. J. Cancer*, 1953, v7, 482.
5. Rous, P., Kidd, J. G., *J. Exp. Med.*, 1941, v73, 365.
6. Mackenzie, I., Rous, P., *ibid.*, 1941, v73, 391.
7. Berenblum, I., Shubik, P., *Brit. J. Cancer*, 1947, v1, 379.
8. ———, *ibid.*, 1947, v1, 383.
9. ———, *ibid.*, 1949, v3, 109.
10. Shubik, P., *Cancer Res.*, 1950, v10, 713.
11. Della Porta, G., Shubik, P., Dammert, K., Terracini, B., *J. Nat. Cancer Inst.*, 1960, v25, 607.

Received Ocober 26, 1961. P.S.E.B.M., 1962, v109.

Partial Protection of Mice by Human Gamma-Globulin Against *Staphylococcus aureus* on Subcutaneous Sutures.* (27090)

JAMES H. TAUBLER, STUART MUDD AND THEODORE SALL
Departments of Microbiology and Public Health and Preventive Medicine, University of Pennsylvania, and U. S. Veterans Administration Hospital, Philadelphia, Pa.

Despite the world-wide challenge of staphylococcal infection, its mechanisms of pathogenesis are not well understood, and there is a dearth of measurable parameters for gauging specific resistance, particularly in man (1). Transfer of observations on staphylococcal infection from the experimental animal to man is subject to serious ambiguities(2). Elek and Conen(3) elaborated a test involving threading of infected sutures through the skin, which they applied to human subjects. James and MacLeod(4) have applied a slight modification of Elek's technic to experimental work in mice in the hope that this would provide a model system less artificial than some other procedures currently in use. In the present study we have examined some effects of pooled human gamma globulin in modifying response to infected sutures in mice, following the procedure of James and MacLeod. It would appear that this system of infected sutures is indeed a useful model.

* This work was supported in part by research grants from Nat. Inst. Health, U. S. P. H. S., and from Veterans Admin. Central Office Research Service, Washington, D.C.