la souche tumorale par repiquages successifs en culture d'organes.

Dans le cas des autres organes, les cellules tumorales se multiplient au contact de l'explant de Poulet, formant un amas compact; elles s'infiltrent dans les tissus suivant la direction des strates conjonctives ou des tuniques musculaires. Dans les organes qui présentent un cloisonnement conjonctif, comme les gonades, l'invasion se fait le long des travées conjonctives. A la faveur de ces voies de pénétration, les cellules tumorales envahissent bientôt tout l'organe, en particulier le cortex ovarien, où elles ont tendance à prendre la place des ovogonies.

Ces résultats montrent qu'on peut cultiver et propager des cellules tumorales sur des organes explantés *in vitro*. Cette méthode donne la possibilité d'étudier les conditions de l'invasion cancéreuse d'un organe, indépendamment de la circulation et de toute connexion. Elle permet d'aborder, dans des conditions de simplicité qui ne sont jamais réalisées dans un organisme, les problèmes des affinités, de la stimulation et de l'inhibition des cellules tumorales. Elle peut orienter les recherches sur la sensibilité différentielle à certaines substances des cellules cancéreuses et des tissus qu'elles parasitent.

E. WOLFF

Laboratoire d'Embryologie expérimentale, Collège de France, Paris, le 25 avril 1956.

Zusammenfassung

Setzt man in Organkulturen *in vitro* kleine Teilstücke eines S180-Maustumors mit embryonalen Organen des Huhnes zusammen, so wandern die tumoralen Zellen in die explantierten Organe über, bevölkern sie und vermehren sich aktiv.

Gewisse Organe wie Vorniere, Mesenterien, Darmwand, Kapsel der Leber, Haut, erweisen sich als besonders geeignet für die Einwanderung und die Vermehrung der tumoralen Zellen.

Tumor Necrosis After the Intra-Tumor Injection of Chemical Agents and Colloidal Radioisotopes

In laboratory and clinical investigations of the use of radioisotopes for the management of tumors, colloidal radioisotopes are injected directly into the tumor[1] with the objective of producing a maximum of tumor necrosis. In view of the increasing number of anti-tumor chemicals which have been successful in producing temporary clinical remission of neoplastic diseases[2], we have undertaken an exploratory study of the ability of a series of those agents to produce tumor necrosis after intratumor injection[3].

Sarcoma 180 tumors were injected on the 8th day after transplantation[4] to the axillary region of Swiss mice. The injectate was a freshly prepared solution or suspension of the agent in saline; each milliliter contained 50 mg of carbon black[7] to mark the intratumor site of deposition. For each agent, 0·03 ml was injected aseptically into each of 5 tumors. After the agent had remained in the tumor for the number of days shown in the Table, the animals were sacrificed and the tumors excised. The tumors were fixed in dilute formalin, sliced to find the region of maximum deposition of carbon black, and hematoxylin and eosin slides of sections from this region were prepared.

The extent of gross tumor necrosis was then determined by microscopic examination, with the absence of stained cell nuclei as the criterion for gross necrosis. This criterion was selected because of the objectivity and relative ease of determining the per cent of the tissue area in which the nuclei did not stain. Inasmuch as there was some variation in size of the injected tumors (within the range from 1·0 to 1·5 cm), the per cent necrosis was calculated on the basis of the necrosis in the 1·0 cm circle centering on the site of maximum car-

Sarcoma 180 Necrosis after Intra-Tumor Injection of Agents

Agent	mg per ml	Days	% Necrosis
methyl nitrogen mustard	0·07	1	48 ± 3
methyl nitrogen mustard	0·67	1	65 ± 4
methyl nitrogen mustard	0·57	3	81 ± 9
β-naphthyl nitrogen mustard[5]	50	3	90 ± 4
phenyl nitrogen mustard[6]	50	3	62 ± 4
p-chlorophenyl nitrogen mustard	50	3	69 ± 5
triethylene melamine	0·40	1	68 ± 2
triethylene melamine	6·7	1	80 ± 4
triethylene melamine	0·67	3	79 ± 7
triethylene phosphoramide	10	1	51 ± 2
triethylene phosphoramide	10	3	85 ± 6
yttrium 90 fluoride	25[a]	3	51 ± 7
chromic radiophosphate	5[a]	3	5 ± 3
cortisone acetate	50	2	14 ± 3
hydrocortone	20	2	42 ± 6
testosterone propionate	25	2	31 ± 4
methyl testosterone	10	3	42 ± 7
diethylstilbestrol	10	3	55 ± 6
estinyl estradiol	15	3	54 ± 5
aminopterin	3·3	2	20 ± 5
furadroxyl	67	2	38 ± 6
isoniazid	50	3	53 ± 6
p-aminosalicylate	67	2	4 ± 2
6-mercaptopurine	67	1	68 ± 2
6-mercaptopurine	83	3	84 ± 6
zinc peroxide	333	2	48 ± 5
p-menthane hydroperoxide	[b]	1	64 ± 2
p-menthane hydroperoxide	[b]	3	87 ± 4
colchicine	2·5	2	28 ± 4
urethane	67	1	69 ± 4
urethane	250	3	83 ± 5
myleran	0·8	1	20 ± 4
controls	saline	1	3 ± 2
controls	saline	3	3 ± 2

[a] Radioisotope concentrations are in millicuries per ml of injectate.
[b] 0·03 ml of liquid *p*-menthane hydroperoxide.

[1] R. H. FLOCKS, H. D. KERR, H. B. ELKINS, and D. A. CULP, J. Urol. *71*, 628 (1954).

[2] C. C. STOCK, in: *Advances in Cancer Research*, Vol. 2 (Greenstein and Haddow, Academic Press, Inc., New York 1954), p. 426.

[3] Presented at the National Meeting of the American Association for the Advancement of Science, Berkeley, California, December 1954.

[4] The original transplant material was donated by Dr. JOHN B. FIELD, Cancer Chemotherapy Laboratory, University of Southern California.

[5] Donated by: Dr. W. C. J. Ross, The Chester Beatty Research Institute, The Royal Cancer Hospital, London, S.W.3.

[6] M. E. MORTON, Nucleonics *10*, 92 (1952). – S. W. MAYER and M. E. MORTON, *Proceedings of the O.R.I.N.S. Conference on Rare Earths in Medicine* (1955) (in press).

[7] Sterling MT grade, Godfrey L. Cabot, Inc., Boston 16, Massachusetts.

bon deposition. Accordingly, the per cent necrosis data on the Table refer to gross necrosis that would be produced in a 1 cm tumor. The standard deviations are also tabulated.

As controls, a total of 24 tumors were injected with 0·03 ml of isotonic saline containing the usual proportion of carbon black. 12 tumors were excised after 1 day, 12 after 3 days. The average tumor necrosis produced by the saline injections was 3% with a standard deviation of 2%, for both the 1 day and 3 day time intervals.

Similar measurements of the necrotic effect of intratumorally injected methyl nitrogen mustard were carried out on Jensen sarcoma, in Sprague-Dawley rats. Three-hundreths of a milliliter of injectate, containing 0·67 mg per ml, were injected into each of 12 tumors. The average necrosis was 70% after 1 day, and 90% after 3 days (for the remaining 6 tumors).

Although the 3 aromatic nitrogen mustards listed in the Table were insoluble in saline, their effectiveness was comparable to the soluble agents. Also, β-naphthyl nitrogen mustard[8] and triethylene melamine, compounds which are normally administered orally to patients, were not less effective after intra-tumor injection than the two compounds which are generally administered intravenously, methyl nitrogen mustard and triethylene phosphoramide. Diethylstilbestrol and 6-mercaptopurine, which also have low solubility in saline were, nevertheless, relatively effective in producing tumor necrosis. The latter 2 compounds and β-naphthyl nitrogen mustard were individually tested for their ability to control the growth of Jensen Sarcoma in rats. Six tenths milliliter of a 50 mg/ml suspension of each compound were injected throughout the tumor on the 6th day after transplanting, and at three-day intervals thereafter. Each compound succeeded in keeping the tumor size down to 15–30% of the controls, and produced apparent disappearance of the tumor in some instances within 2 weeks—with survival periods longer than 6 months.

The intra-tumor administration of insoluble anti-tumor compound could reduce the systemic toxic effects frequently observed after the systemic administration of anti-tumor agents, since insolubility would ordinarily prevent rapid movement from the tumor. Of course, such administration of insoluble agents would be limited to the tumors for which radioisotopes have been used—bronchogenic, breast, prostate, cervical and other types of accessible tumors (and possibly for control of ascitic fluid formation). In principle, it would appear desirable to investigate the effectiveness of intra-tumor combinations of radioisotopes with one or more anti-tumor chemicals of the nitrogen mustard, hormone or anti-metabolite types.

S. W. Mayer and M. E. Morton

Radioisotope Research Unit, Veterans Administration Hospital, Long Beach, California, and the School of Medicine, University of California at Los Angeles, April 23, 1956.

Zusammenfassung

Mehrere klinisch wirksame, geschwulsthemmende Stoffe verursachten relativ rasch Nekrosen der Mäusesarkoma-180-Tumoren, wenn sie, entsprechend der klinischen Anwendung von radioaktiven Kolloiden, direkt in den Tumor injiziert wurden. Sowohl unlösliche, wie lösliche Verbindungen ergaben Nekrose. In den Tumor injiziertes 6-Merkaptopurin, Diäthylstilböstrol und β-Naphtyl-Stickstoff-Lost verminderten alle bei Ratten das Wachstum des Jensen-Sarkoms.

[8] A. A. Videbaek and S. Kaae, Acta med. Scand. *149*, 361 (1954).

Potentiation Action of Ibogaine (Bogadin TM) on Morphine Analgesia

Neostigmine[1], B-diethylaminoethyldiphenylpropylacetate hydrochloride[2], certain adrenocortical hormones such as DCA[3], and insulin[4] have been shown to potentiate morphine analgesia. Chlorpromazine prolongs the analgetic morphine effect[5] but does not necessarily enhance it[6]. Therefore, the reduction of morphine analgesia in mice pretreated with reserpine was a rather unexpected finding[5]. The combination effects of these phrenotropic agents with morphine prompted us to investigate the pharmacodynamic interactions of morphine with ibogaine, an indole alkaloid from *Tabernanthe iboga*[7] with characteristic central stimulant properties[8].

Potentiation of Analgetic Effect of Morphine Sulfate by Ibogaine HCl

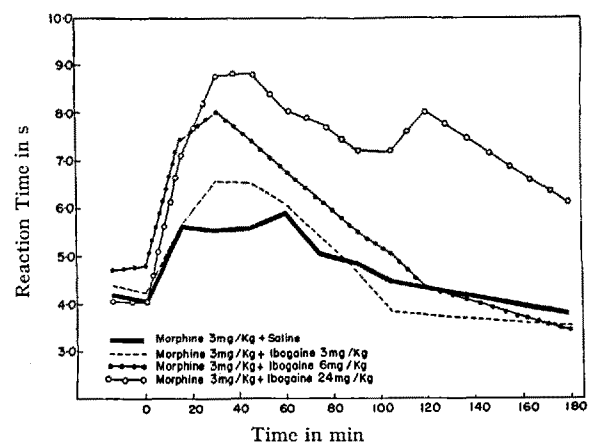

Fig. 1.—Comparison of average reaction time to a thermal pain stimulus applied to the mouse tail following subcutaneous injection of morphine alone and in combination with ibogaine. Each curve represents an average from a group of 10 mice. Prolongation of the reaction time following the combination of morphine and ibogaine becomes more pronounced as the dose of ibogaine increases.

Methods.— 370 female white mice (Webster strain) were tested for their reaction to pain by directing a beam of heat on the tip of their tails (Gross[9]). The reaction time was measured for each mouse as determined by the characteristic tail flick. Double readings ob-

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