Yet the large excess of serum in these cases might logically be expected to compete for the toxin and thus reduce the hemagglutination titre. If normal serum proteins were in some way "bound" at the red cell's surface antitoxin might be similarly bound. The addition of toxin to a red cell-antitoxin mixture should then be expected to result in agglutination. Actually a mixture of sheep cells and horse antitoxin incubated for an hour before addition of toxin remains unagglutinated. Finally, the presence of serum at the cell surface should show some, if slight, toxin adsorption if such serum were actually responsible for agglutination. An experiment was performed in which 460 millions of sheep red cells were mixed with only 200 mouse MLD of toxin at 10°C. Putnam *et al.*[5] have determined that the number of molecules per LD_{50} is of the order of 2.1×10^7. An MLD would contain less than twice as many molecules.[6] Thus in the mixture used there was only of the order of 18 toxin molecules per cell. The adsorption of only a few molecules per red cell would have resulted in a large percentage adsorption of toxin. Yet when the red cells were centrifuged down and the supernate titrated no significant reduction of toxic titre was found.

Summary. Type A botulinal toxin causes agglutination of chicken, guinea pig, rabbit, sheep and human red cells. The agglutination is not accompanied by evidence of adsorption of the toxin by the red cells. Temperature, particularly in the case of sheep cells, influences the hemagglutination titre. When washed free of toxin, agglutinated chicken cells tend to remain agglutinated while agglutinated sheep cells are more readily dispersed.

[5] Putnam, F. W., Lamanna, C., and Sharp, D. G., *J. Biol. Chem.*, 1946, **165**, 735.

[6] Lamanna, C., McElroy, O. E., and Eklund, H. W., *Science*, 1946, **103**, 613.

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Regression and Reabsorption of Mammary Tumors by Extracts of Degenerating Amphibian Skin.

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The chemotherapy of cancer has received and still receives considerable attention. Most work has been directed towards the examination of substances which inhibit growth as contrasted with those which are curative in the sense of bringing about the complete regression of the tumor. The works of Roffo[1] and of Boyland[2] were of especial interest to the writer and led to the experiments here described. Roffo first found that the injection of fresh extracts of striated muscle into rats implanted with a transferable fibro-sarcoma 10-19 days previously were without inhibitory or regressional effects. The same negative findings were recorded when tumorous tissue was tested *in vitro*. However, striking regression and disappearance of tumorous tissue was obtained when applying hydrolysates of striated muscles both *in vivo* and *in vitro*. Boyland was able to demonstrate less striking results on grafted sarcomata and spontaneous carcinomata in mice following oral administrations of acid extracts of ox heart muscle. Pronounced inhibition of tumor growth was obtained but no cases of complete regression.

Reasoning from a general hypothesis that degenerating tissues might, in certain cases, contain a substance or substances detrimental to the growth or persistence of tumorous tissue, experiments were planned involving

[1] Roffo, A. H., *Biol. Inst. Med. Exp.* (Buenos Aires), 1938, **14**, 257.

[2] Boyland, Eric, *Biochem., J.*, 1941, **35**, 1283.

TABLE I.
Effect of Skin Extracts and Control Solutions on Mammary Tumors in Mice.

Case No.	Animal No.	Extract or control solution	Dosage (cc)	No. of injections	Original size of tumor* (mm)	Size 2 days after last injection† (mm)
1	Cn 1	Cntr.	0.3	6	34	33/12
2	Cn 2	Cntr	0.3	6	37	22/12
3	Cn 3	Cntr.	0.3	6	40	29/12
4	CF 1	CS	0.3	6	29	0/12
5	CF 2	CS	0.3	6	33	4/12
6	CF 3	CS	0.3	6	31	7/12
7	CF 4	CS	0.3	6	30	0/12
8	Cn 4	Cntr.	0.15	6	26	24/12
9	Cn 5	Cntr.	0.15	6	24	14/12
10	Cn 6	Cntr.	0.15	6	25	16/12
11	CF 5	CS	0.15	6	22	7/12
12	CF 6	CS	0.15	6	17	0/12
13	CF 7	CS	0.15	6	24	0/12
14	Cn 7	Cntr.	0.05	9	30	32/18
15	Cn 8	Cntr.	0.05	9	21	17/18
16	CF 8	CS	0.05	9	20	0/18
17	CF 9	CS	0.05	9	29	14/18
18	CF 10	CS	0.05	9	31	11/18
		Subcutaneous Injections.				
19	Cn 9	Cntr.	0.3	9	35	40/18
20	CF 11	CS	0.15 and 0.3‡	9	25	22/18
21	CF 12	CS	0.15 and 0.3‡	9	29	22/18
22	CF 13	CS	0.3	9	40	38/18

* Length plus breadth.
† The numerator = length plus breadth; denominator = number of days since first injection.
‡ Five injections of 0.15 cc followed by four of 0.3 cc.

the effects of extracts of degenerating amphibian tail muscle and skin on spontaneous tumors in mice.

Materials and methods. Extracts of normal and degenerating anuran larval tail muscle and skin were prepared. Preliminary tests showed that those of degenerating skin were most effective. These (Extract CS) were prepared as follows: *R. catesbeiana* larvae were selected in a metamorphic stage at which tail resorption was well underway. The tail integuments of approximately 60 larvae were stripped off and ground to a homogeneous consistency in a mortar. 25 cc of cold 5N HCl was added to 5 cc of the ground skin and the mixture allowed to stand, at room temperature, for 48 hours. The mixture was then filtered several times and the final filtrate neutralized against litmus with dry $NaHCO_3$. A small amount of sterile distilled water was now added to bring the volume up to 25 cc. The extract was then placed in a sterile bottle and refrigerated until ready to use. Neutral control solutions, for injection, were also prepared with 5N HCl and $NaHCO_3$.

CFl strain mice* of uniform age and showing definite surface indications of early spontaneous mammary tumor growth were utilized for injection purposes. The mice selected for injection were placed in individual cages and maintained under uniform diet conditions for at least a week prior to use in the tests. The selection of individuals for extract and control solution injection purposes was made on the basis of general healthiness of the animals and similarity in tumor size and location. Extracts and control solutions were injected in most cases directly into the tumors and in a few cases subcutaneously on the back. The injections were made every other day.

Results. The results are listed in Table I. They include only those animals which lived for more than 10 days following the last injection. Such mortality (27%) as occurred was

* The mice were supplied by Carworth Farms, New City, N. Y.

distributed quite equally among extract and control injected and non-injected stock animals. The mortality was attributed entirely to extreme fluctuations in room temperature beyond control of the writer. The injected animals lived frequently for 30 or more days following the last injection. In certain individuals no new tumors appeared. In others one or two developed but never in close proximity to the injection site. For most cases involving tumor regression the effect was noted within 3 to 6 days, after which reduction in size progressed steadily.

In 6 cases (not listed in Table I) injections were terminated when the tumors were reduced approximately 50%. In 4 of these, tumor regression continued, but at a lowered rate, while in the other 2 regression ceased shortly and the tumors enlarged subsequently. New tumors appeared in several of the cases listed in Table I at the same time the injected tumors were undergoing reduction in size. This was not true, however, of any of the subcutaneously injected animals.

A study of the results listed in Table I will indicate that tumors showed definite regression following injections of control solutions in doses of 0.3 and 0.15 cc while 0.05 cc injections were relatively ineffective. Extract injected tumors, however, evidenced much greater regression in rate and size in all dosages used and accounted for the five cases of complete resorption.

Histological studies were made at successive growth stages of normally developing CFl strain mammary tumors and also of tumors undergoing degeneration as the result of control solution and skin extract injections. The normal stock tumors showed the usual transformations from simple adenomatous to malignant carcinomatous types. Tumors injected several times with control solutions usually showed cystic development localized in one or more regions of the growth with large masses of carcinomatous tissue and some adenomatous in the remaining regions. In contrast, extract injected tumors after several injections evidenced larger and more numerous cysts accompanied by considerable amounts of adenomatous but very little carcinomatous tissue.

The subcutaneous injection of skin extract and control solution (Table I, cases 19-22) is of interest in that inhibition of tumor growth and slight regression in size is suggested. The number of cases is too small, however, to justify any definite conclusion.

Summary. Neutralized acid extracts of degenerating tail skin from involuting *R. catesbeiana* larvae were injected into rapidly growing spontaneous mammary tumors in mice. Marked regression of the tumors was obtained in all instances resulting in complete disappearance of all tumorous tissue in 5 out of 13 cases within eight to eighteen days after the initial injection.

The application of neutralized acid control solutions, similarly injected, resulted with one exception in slight to marked reduction in tumor size but never in complete resorption.

The tumors of one control and 3 extract animals, subcutaneously injected, showed only slight regression in size but are indicative in that growth was apparently inhibited.

The results indicate that a neutralized HCl acid extract of degenerating anuran tail skin contains some substance or substances possessing inhibitory and degenerative effects on spontaneous mammary tumors in mice beyond those exhibited by a neutralized acid control solution similarly injected.