war work in some other part of the country and who wish to return to their original jobs and homes should be considered as drafted men and women returning from service to their country. Every effort should be exerted to make their former jobs available to them. They should be furnished with severance pay sufficient to get them back to their homes and over the initial hard times. This is just as much a national responsibility as that recognized in giving "mustering-out pay" to members of the armed forces.

SPECIAL ARTICLES

CHEMOTHERAPY OF FILARIASIS IN THE COTTON RAT BY ADMINISTRATION OF NEOSTAM

FLORIDA cotton rats are frequently infected with a filarial worm, *Litmosoides carinii*. The adult parasites dwell in the pleural space and microfilariae occur constantly in the peripheral blood of the rats. Since infected animals can be readily procured and since the infection bears some similarity to certain of the human filarial diseases, the cotton rat filariasis appears to supply a much-needed means of testing drugs for potential activity in the treatment or prophylaxis of human filariasis.

Several drugs have been tested in this laboratory for therapeutic action in the cotton rat infection. Among these, neostam (stibamine glucoside, Burroughs Wellcome and Co.) has given particularly favorable results. The adult filarial worms have been killed after a few doses of this drug and gradually thereafter microfilariae have disappeared from the peripheral blood of treated animals.

In Table 1 are given data on ten treated and two control untreated cotton rats. Four doses of neostam, each of from 40 to 60 mgm, were administered intramuscularly to the animals every week until autopsy and microfilaria counts on the tail blood were made almost every day. The animals were autopsied after the intervals indicated in the table and searched for adult worms. In the treated rats presented in the table, in which the microfilaria count finally reached zero, every adult worm was dead when recovered at autopsy. Usually the adult worms from the treated rats were found massed together, often completely enveloped by inflammatory exudate. In other treated rats, besides those shown in the table, which were autopsied before all microfilariae disappeared from the peripheral blood, the adult worms were likewise dead and enveloped by exudate, in some animals after as brief a time as eleven days from the beginning of treatment. It appears from these data that the repeated injection of neostam has resulted in the cure of filariasis in the cotton rat and, since the drug is well tolerated by man in comparatively large doses,[1] its trial in human cases of filariasis seems to be indicated.

TABLE 1

EFFECT OF NEOSTAM ON THE FILARIAL WORM LITOMOSOIDES CARINII IN COTTON RATS

Cotton rat No.	Microfilariae counted in 100 microscope fields × 430 on designated days											Adult worms recovered at autopsy†
	Before treatment	After treatment										
		1	7	14	21	28	35	42	49	56	64	
1	136	94	100	52	20	28	16	7	5	2	0*	40 to 50; dead; matted together.
2	44	0	4	3	5	5	1	3	1	0*		5 to 10; dead; enveloped by exudate.
3	50	28	32	22	24	28	4	1	3	1	0*	10; dead; matted together.
4	4	4	0	0	0	0	0*					1; dead.
5	12	10	0	0	0	0	0*					10; dead; enveloped by exudate.
6	92	62	92	70	38	7	6	6	0	3	0*	50; dead; some matted together.
7	180	152	230	84	16	64	8	3	2	1	0*	50; dead; matted together.
8	92	72	16	4	0	0	0*					25; dead; enveloped by exudate.
9	124	44	56	0	0	0*						20; dead; enveloped by exudate.
10	108	96	116	62	26	5	0*					40; dead; some matted together.
11 (Control)	16	18	36	12	24	20	10	38	48	42	52*	8; living; freely moving.
12 (Control)	252	232	232	176	110	192	90	176	136	186	198*	50; living; freely moving.

* Day of autopsy.
† When worms are matted together, numbers are approximated.
Schedule of treatment: Rats 1 through 7: 40 mgm; Rats 8 through 10; 60 mgm } 4 times weekly until autopsy.
Rats 11 and 12: Untreated controls.

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THE ROLE OF CALCIUM IN CARCINOGENESIS

IN a comprehensive review on the role of the fixed bases in cancer, Shear[1] pointed out that "much con-

[1] L. E. Napier, *Indian Jour. Med. Res.*, 16: 911, 1929.
[1] M. J. Shear, *Am. Jour. Cancer*, 18: 924, 1933.

fusion exists as regards the role in cancer of the commonly occurring constituents, sodium, potassium, calcium and magnesium."

In our integrated program on epidermal methylcholanthrene carcinogenesis in mice quantitative determinations of the fixed alkalies and of iron in normal, in benzene-treated and in methylcholanthrene-treated epidermis have been carried out.[2] These investigations showed that one application of methylcholanthrene reduced within 10 days the epidermal calcium and iron content to 50 per cent. of the normal, benzene alone being without effect. Tri-weekly treatments of the epidermis with the carcinogen for 60 days did not cause a further significant decrease in the calcium content, although the epidermal hyperplasia was more extensive than after a single application at 10 days. The next step was to determine the content of this metal in a carcinoma derived from mouse epidermis. For this purpose a rapidly growing transplantable carcinoma,[3] produced in the skin of a Swiss mouse by methylcholanthrene, was used. The methods for the determination of calcium[4] and for nucleoprotein phosphorus, (N.P.P.) the basis of reference, have been given.[5] For analysis the carcinomas were cleaned of adhering blood and connective tissue after removal and then cut in small pieces and thoroughly mixed before sampling. A small piece of each tumor used for analysis was fixed for microscopic examination to determine the extent of necrotic tissue present.

Since transplantable tumors may contain variable amounts of necrotic tissue and since such necrotic tissue is known to be rich in calcium,[1] it was necessary to exclude as far as possible the presence of necrotic tissue. This was accomplished by using young tumors, 10 to 14 days after inoculation, at which time they are small, solid, and contain little, if any, necrotic material. Six to 12 tumors were required for a single analysis.

The calcium content was determined in nine different samples. The results, together with those of normal, benzene-treated and epidermis, rendered hyperplastic by the carcinogen, are shown in Table 1. The Ca/N.P.P. ratio of hyperplastic epidermis was 50 per cent. less than normal, and that of the carcinoma about 50 per cent. less than the hyperplastic epidermis. The calcium content of the carcinoma varied from 0.007 to 0.014 mg calcium per 100 mg tumor.

With the microincineration technique Paletta, Cowdry and Lischer[6] found demineralization in both benign and methylcholanthrene, hyperplasia, particularly in the distal part of the spinous layer. With the same method Scott[7] reported that hyperkeratosis, warts, human breast and skin carcinomas showed much less calcium and magnesium in their cytoplasm than did similar normal types. However, it is not possible to distinguish between calcium and magnesium by the microincineration technique.

TABLE 1

Tissue	No. of analysis	Mg. calcium per 100 mg tissue average	Mg. nucleoprotein phosphorus per 100 mg tissue	$\frac{\text{Calcium}}{\text{Nucleoprotein phosphorus}} \times 10$
Normal epidermis	6	0.044	0.118	3.60
Benzene-treated epidermis	7	0.042	0.125	3.40
Methylcholanthrene-treated epidermis (hyperplastic)	18	0.019	0.130	1.46
Carcinoma	9	0.009	0.121	0.75

The extent to which the presence of necrotic tissue affects the calcium content was determined by the analysis of the same strain of transplantable tumor in the groups (Table 2) of older tumors in which necrotic tissue was visible to the naked eye. In Group B, C and D this poorly visible necrosis was verified histologically.

TABLE 2

	Mg Ca per 100 mg tumor Average	Ca/N.P.P. × 10 Average
A. Large necrotic tumors	0.077	
B. Large necrotic tumors freed of gross necrotic material	0.042	3.42
C. Tumors about 15 mm in diameter freed of gross necrotic material	0.029	2.49
D. Tumors about 10 mm in diameter freed of gross necrotic material	0.019	1.61

These results show the effect of including necrotic material in the tissues analyzed and the importance of excluding it in determination of the calcium content of the actual neoplastic tissue.

SUMMARY

Estimations of the calcium content of the mouse epidermis during the process of experimental carcinogenesis reveal two distinct phases: an immediate reduction in the calcium content which persists at a fairly constant level for many weeks and a further reduction when the epithelial cells have been transformed into cancer cells. Reduction of calcium in the

[2] C. Carruthers and V. Suntzeff, *Cancer Res.*, 3: 744, 1943.

[3] The authors are indebted to Dr. Z. K. Cooper of this hospital for the carcinoma.

[4] V. Suntzeff and C. Carruthers, *Cancer Res.*, 3: 431, 1943.

[5] C. Carruthers and V. Suntzeff, *Jour. Nat. Cancer Inst.*, 3: 217, 1942.

[6] F. X. Palletta, E. V. Cowdry and C. E. Lischer, *Cancer Res.*, 1: 942, 1941.

[7] G. H. Scott, *Biological Symposia*, 10: 277, 1943.

hyperplastic epidermis is an important feature in this experimentally induced precancerous condition.

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SURVIVAL OF NORMAL CELLS IN PENICILLIN SOLUTIONS LETHAL TO MALIGNANT CELLS

STUDIES at the Wistar Institute of Anatomy and Biology, made possible through the kindness of Dr. M. R. Lewis and Dr. W. H. Lewis,[1] have revealed a selective lethal effect of penicillin upon rat and mouse sarcoma cells, of which a full account will be published later.

In roller tube cultures, untreated sarcoma cells, grown with normal fibroblasts derived from explants of muscle from the same strain of tumor host, grow fully as vigorously as the normal cells. However, upon addition of penicillin (Squibb's sodium salt of penicillin), the sarcoma cells were selectively damaged. With proper choice of the dosage level, it was found possible to kill all the sarcoma cells without damaging the normal fibroblasts. Higher dosage damaged the non-malignant cells, but the dose required was two to three times that required to produce an equivalent injury in malignant cells.

Damage was arbitrarily classified as incipient (granularity of 50 per cent or more of the cells, and a faintly withered appearance of the cell membrane), marked damage (rounding, coagulation or disintegration of the cells, short of 100 per cent), and lethal (no living cells visible). Table 1 shows the totals of

TABLE 1

NUMBERS OF EXPLANTS OF SARCOMA CELLS AND OF NORMAL FIBROBLASTS SHOWING DIFFERENT GRADES OF DAMAGE. COMBINED TOTALS OF ALL EXPERIMENTS.

	None	Incipient	Marked	Lethal	Totals
Normal	112	37	57	0	206
Sarcoma	0	29	156	78	263

explants classified according to damage shown. These results include four induced rat tumors and one induced mouse tumor. Another induced mouse tumor, not included in the data of Table 1, did not show a definite selective response.

In twenty-five experiments, the treated tumor cultures were implanted into rats of the corresponding 100 per cent-susceptible strain. All cultures graded as "lethal damage," and most of those graded as "marked damage" failed to produce tumors, whereas the untreated cultures produced tumors.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A RAPID, QUANTITATIVE METHOD FOR THE DETERMINATION OF PENICILLIN

THE following assay method affords a rapid, convenient and relatively accurate method of determining the potencies of antibiotic substances in terms of suitable standards. Since assays may be completed during the course of a working day, the method is particularly useful as a guide to the time of maximum production of penicillin, for the control of isolation procedures of various antibiotic agents, in the determination of the amount of deterioration and for related problems.

Earlier experiments[1] on the use of filter paper as a matrix to support mold growth led to the development of the present assay method in which the antibiotic substance to be assayed is placed on sterile, absorbent paper discs on the surface of inoculated nutrient agar. The function of the paper is to act as a reservoir from which the antibiotic substance diffuses into the agar where it inhibits the growth of the test organism. This results in a clear zone surrounding the disc. It was found that the diameter of this zone of inhibition is proportional to the amount of antibiotic substance present and, for practical purposes, may be considered a straight-line function of the log concentration.

The following example illustrates the simplicity of the assay. Nutrient agar,[2] approximately pH 7.0, was seeded with *B. subtilis* spore suspension[3] so as to contain approximately 2×10^5 spores per cc and 25 cc portions of this medium were pipetted into 90 mm petri dishes (depth of the agar approximately 4 mm). Four sterile, filter paper discs, diameter 15.3 mm,[4] were evenly spaced upon the agar. By means of a

[1] We are indebted to the Carnegie Institution of Washington Department of Embryology and to the International Cancer Research Foundation, as well as to the Wistar Institute of Anatomy and Biology, for aid in carrying out this work.

[1] M. B. Sherwood, *Jour. Bact.*, 43: *Proc.*, 779, 1942.

[2] G. L. A. Ruehle and C. M. Brewer, *U. S. Dept. of Agriculture Circ.* 198, 1929.

[3] J. W. Foster and H. B. Woodruff, *Jour. Biol. Chem.*, 148: 723, 1943.

[4] Discs were cut from E. and D. No. 615 filter paper with a sharpened cork borer.