frequency of nuclei in all stages of the mitotic cycle *i.e.,* resting stage, prophase, metaphase, anaphase and telophase. The proportion of nuclei in anaphase as compared to metaphase, and of both these stages to prophases in chromatin-tested animals was found to be the same as in the controls. It was concluded, therefore, that the observed increase in the frequency of metaphases and anaphases was due to a true increase in the rate of mitosis, and that the phase of the nuclear cycle in which the stimulation occurred was some portion of the resting stage.

To determine whether material from chromatin administered intravenously can become incorporated into the nuclei of the liver, chromatin was prepared from animals which had received P^{32} as Na_2HPO_4 and this injected into test rats 24 hours after partial hepatectomy. Related substances were similarly labeled, isolated and administered to the test animals. The livers were perfused and nuclei isolated 3 hours later. Table 1 shows the accumulation of P^{32} in the liver tissue

TABLE 1

P^{32} IN REGENERATING LIVERS THREE HOURS AFTER INTRAVENOUS INJECTION OF LABELLED SUBSTANCES

Material injected	Number of rats	P^{32}/gm liver as per cent. dose	Standard error of mean
1. Inorganic phosphate	84	4.52	0.083
2. Inorganic phosphate	10	4.36	0.084
3. Rat chromatin	19	26.30	0.670
4. Lipid (rat chromatin) ..	15	32.20	0.390
5. Fat free chromatin (rat)	5	7.10	0.480
6. Adenosine triphosphate .	7	4.43	0.320
7. Rabbit chromatin	8	23.10	0.200
8. Rabbit chromatin soluble in 1 M NaCl	8	16.20	0.470

from the various substances administered. There is a large accumulation from chromatin and from phospholipid which is undoubtedly due to the particulate nature of these materials. Table 2 gives the P^{32} con-

TABLE 2

P^{32} UPTAKE BY NUCLEI

Substance injected	Per cent. dose gm nuclei	Per cent. liver P^{32} in nuclei
Inorganic phosphate	1.54	2.1
Rat chromatin	5.08	1.2
Rabbit chromatin	5.06	1.3
Rabbit chromatin soluble in 1 M NaCl	4.23	1.6
Fat free chromatin (rat) .	5.45	4.6
Lipid (rat chromatin) ...	5.15	1.0

centration in the liver nuclei as per cent. injected dose per gram nuclei, and also the nuclear P^{32} as per cent. of the total liver P^{32}. From the latter figures it is clear that P^{32} from chromatin becomes incorporated into liver nuclei most rapidly from fat-free chromatin, least rapidly from lipid, with inorganic phosphate and crude chromatin in an intermediate position. The greater concentration of P^{32} in nuclei from animals receiving chromatin can not therefore be attributed to the mere accumulation of the particulate matter in the liver. The P^{32} from the chromatin evidently does not enter the cellar nucleus as phosphate ion but as a compound that may be of considerable size and complexity. The results are in some respects analogous to those of previous experiments which showed that the nucleoprotein of the living nucleus is in a state of dynamic equilibrium in which portions of it are constantly being removed and replaced.[3]

That the mitosis stimulating property of chromatin is not confined to the liver is indicated by preliminary experiments with standardized skin wounds of the rat. When a saline extract of fat free chromatin was applied locally, granulation tissue appeared 2 to 3 days earlier than in the controls, and also filled the wound area sooner. An excess of granulation tissue was not formed and there was no evidence of irritation or inflammation.

CONCLUSIONS

(1) Chromatin contains some factor which stimulates the rate of mitosis in the liver.

(2) Radioactive phosphorus becomes incorporated into nuclei of regenerating liver more rapidly from fat free chromatin than from phospholipid or inorganic phosphate.

(3) Since phosphorus containing compounds of considerable size and complexity may enter the cell and become built into the nucleus, the results suggest a possible means whereby controlled gene mutations may be produced if the gene be considered a nucleoprotein complex. The replacement of a portion of the nucleic acid or nucleotide by another of different structure may produce a change in function in the region of the chromosome so effected. Changes in type of pneumococcus[5] may be accounted for by such a process, rather than by alteration of the cytoplasm, as has been suggested.[6]

(4) Preliminary results indicate that a derivative of chromatin stimulates the rate of formation of granulation tissue in skin wounds.

Details of these experiments will be published elsewhere.

ALFRED MARSHAK
A. C. WALKER

RADIATION LABORATORY,
UNIVERSITY OF CALIFORNIA

SURVIVAL OF THE MAMMARY TUMOR MILK AGENT OF MICE[1, 2]

PREVIOUS observations demonstrated that the milk agent or inciter of mammary tumor in mice, normally

[5] O. T. Avery, C. M. MacLeod and M. McCarthy, *Jour. Exp. Med.*, 79: 137–157, 1944.

[6] T. H. Sonneborn, *Proc. Nat. Acad. Sci.*, 29: 338–343, 1943.

[1] Preliminary report.

transferred by nursing,[3] may be obtained from either spontaneous[4] or transplanted[5] mammary carcinoma or from lactating mammary tissue[6] or whole blood.[7] The active agent[8, 9] has also been found in cell-free filtrates. In this report preliminary data are presented from further studies on the characteristics of the active agent.

In these studies, tests for the presence of the tumor agent were conducted by the inoculation of female mice which had parents that did not transfer the influence in their milk but which had the inherited susceptibility for spontaneous mammary cancer. They were inoculated when they were 4 to 5 weeks of age. Following inoculation, they were permitted to breed regularly to insure an adequate hormonal stimulation. The mice were either ABC or ZBC animals. The ABC mice were produced as follows: females of the C57 black (B) and males of the A strain were mated, and the hybrid females produced were crossed to males of the A stock. The resulting back-cross progeny were called ABC animals. The ZBC mice, similarly, were back-cross animals to the Z or C3H stock following the mating of hybrid females produced by reciprocal matings between the descendants of the fostered mice of the A and Z or C3H stocks. To date, the incidence of mammary cancer in the controls, numbering several hundred, has been less than 1 per cent.

The first group of experiments to be reported was designed to produce additional evidence that the active milk agent may be carried in transplanted mammary cancer. The tumor used as the inoculum had developed in a female of the cancerous C3H stock and was designated as tumor No. 5663. The spontaneous tumor was tested to insure the presence of the agent. In the demonstration of the active agent, the tissue was first macerated and suspended in Ringer's solution (1:10 by volume). After the suspension was centrifuged, 0.5 ml of the supernatant liquid was injected intraperitoneally into each of 10 ABC[10] mice, of which 1 is still living and 8 (80 per cent.) have died with spontaneous mammary cancer. The balance of the supernatant liquid was filtered through a Berkefeld N candle. Eleven mice of the same stock were injected, also intraperitoneally, with 0.5 ml of the filtrate; 8 of these have developed mammary carcinoma.

Tumor No. 5663 was also inoculated into C3H mice and their hybrids which had not been exposed to the active milk agent. Transplants of the tumors were tested for the presence of the agent after the 1st and 10th transplant generations.

After one passage in mice, the tumors were macerated and suspended in Ringer's solution (1:4). The original suspension was injected subcutaneously into ABC mice in a dosage of 0.4 ml. Four of the 11 test animals have developed spontaneous mammary cancer and the others have survived 16 months free from tumors. A portion of the suspension was passed through a tested Berkefeld N filter and the filtrate was injected into 12 ABC mice in a dosage of 0.2 to 0.4 ml. Eight of the mice developed mammary cancer, while 4 failed to develop tumors.

Following the 10th serial passage of tumor No. 5663 in mice that do not carry the active milk agent, the tissue was suspended in distilled water (1:3) and 1 ml was given by mouth (tube-feeding) to each of 22 mice of the ZBC group. Of these, 8 are living at 12 months of age and 12 have died from spontaneous mammary cancer.

These results demonstrate that the mammary tumor milk agent persists in association with tumor transplants carried through 10 serial passages in mice that did not themselves carry the milk influence.[5] This indicates that the agent was continually produced within the transplant tumor cells. The findings seem to provide evidence for the theory, previously advanced,[11] that the active agent may play a role in the production of genetic mutations in transplantable tumors.

Tumor tissue prepared in the manner described above was also utilized in initial studies on chick embryos. Twenty-five hundredths of a milliliter of suspended tumor tissue (1:4 in Ringer's solution) of the first transplants of tumor No. 5663 was injected into the yolk sac of 5-day-old chick embryos. Twelve days later the yolks from 2 eggs that were negative grossly were pooled and 1 ml of the unfiltered yolk was injected intraperitoneally into mice of the ABC stock. Six mice were used, of which 3 developed mammary cancer. The others have developed no tumors during 17 months of observation. These results demonstrated that the milk influence survived 12 days in

2 These studies were supported by grants from the Citizens Aid Society of Minneapolis, the University of Minnesota Graduate School Cancer Research Fund and the Jane Coffin Childs Memorial Fund for Medical Research.

3 J. J. Bittner, SCIENCE, 84: 162, 1936.

4 *Ibid.*, 93: 527, 1941.

5 *Ibid.*, *Bull. Minn. Med. Found.*, 4: 94, 1944.

6 *Ibid.*, *Proc. Soc. Exp. Biol. and Med.*, 45: 805, 1940.

7 G. Woolley, L. W. Law and C. C. Little, *Cancer Res.*, 1: 955, 1941.

8 J. J. Bittner, *Cancer Res.*, 2: 710, 1942.

9 *Ibid.*, SCIENCE, 95: 462, 1942.

10 Had living tumor cells been injected, the ABC mice, being the progeny of hybrids derived from mating mice of the A and C57 black stocks, should not have been susceptible to a C3H tumor. Moreover, transplanted tumors would have developed at the site of injection, intraperitoneally, within a few weeks.

11 J. J. Bittner, *Am. Jour. Cancer*, 36: 44, 1939.

embryonated eggs, possibly in the presence of living tumor cells.

In a second group of experiments, further studies were made on the survival of the milk influence in embryonated eggs. These experiments were performed with a filtrate made from lactating mammary tissue of mice which carried the active agent. Lactating mammary tissue was macerated, suspended in broth (1:10) and centrifuged. The supernatant liquid was then filtered through a tested Berkefeld N filter, was tested for the presence of the active agent, and used to inoculate chick embryos. The test to ascertain whether the filtrate contained the active milk agent was performed by injecting 14 mice with 1 ml of the material intraperitoneally. Mammary tumors have resulted in 6 of the test mice, demonstrating the presence of the active agent. The remaining mice are alive and free from tumors at 13 months of age.

Eggs containing 5-day-old chick embryos were injected with 0.25 ml of the filtrate of mammary tissue. Two eggs were incubated for 1 hour, after which the yolks were collected and pooled, and 1 ml of the unfiltered yolk was injected intraperitoneally into each of 8 mice. Of these, 5 have developed spontaneous mammary tumors while the others are still under observation.

Other eggs of 5 days' embryonation that had received the filtrate were incubated for 12 days, after which time the yolks were pooled. Part of the yolk was centrifuged undiluted at low speed. Of 16 mice that received 1 ml of the unfiltered yolk intraperitoneally, 6 have developed mammary cancer while the others are still under observation. The balance of the yolk from eggs was extracted with Locke's solution (1:3) and centrifuged. The supernatant liquid was filtered through a Berkefeld V filter. One milliliter of the filtrate was injected intraperitoneally into each of 18 mice. Of these, 6 have shown spontaneous mammary tumors and the 12 others are living without growths at 13 months of age.

In the second group of experiments, a filtrate of mammary tissue, proved to contain the active tumor milk agent, did not produce grossly demonstrable tumors in 5-day embryonated eggs after 12 days of incubation. However, both unfiltered and filtered egg yolks, after 12 days' incubation, were found to contain the active mammary tumor milk agent. Therefore, our results, although adduced from a small number of mice, are interpreted to mean that the milk agent survived 12 days in the yolk sac in the absence of living mouse cells.

SUMMARY

The mammary tumor milk agent has been recovered from a transplanted mammary carcinoma that was carried for 10 passages in mice that did not themselves originally have the agent. It is possible that the agent, carried in the transplanted mammary tumor cells, may be responsible for the genetic mutations which have been detected in transplantable tumors.

Preliminary results show that the agent can be recovered from the yolk sac of chick embryos 12 days after the injection into eggs of either tumor suspensions or cell-free filtrates of tissues containing the active agent.

JOHN J. BITTNER
CHARLES A. EVANS
ROBERT G. GREEN

DIVISION OF CANCER BIOLOGY OF THE
DEPARTMENT OF PHYSIOLOGY, AND
DEPARTMENT OF BACTERIOLOGY
AND IMMUNOLOGY,
UNIVERSITY OF MINNESOTA MEDICAL
SCHOOL, MINNEAPOLIS

SIMULATION OF PHOTOPERIODICITY BY THERMOPERIODICITY

A TOMATO plant is photoperiodically indifferent, since under properly controlled temperatures the daily length of illumination has practically no effect on its development. For best growth and fruit set, tomatoes have to be kept warm during day (26° C) and cool during night (15–18° C), which has been called thermoperiodicity.[1] The cool period for optimal development is only effective in darkness or in at least greatly reduced light, so that plants subjected to the proper temperature sequence in continuous light do not set fruit. Since no fruit set is possible above 22° and below 10° night temperature, tomatoes do not bear fruit in winter or spring nor during hot spells in summer, even though day temperatures are within the rather wide range of possible growth (15–35° C). It also has been established that each day sugar production by assimilation in tomato leaves continues only until early afternoon, when a maximum sugar content is reached.[2]

In Southern California winter and early spring night temperatures are usually below 10°, but the afternoon temperatures range between 15° and 20°, optimal for growth and fruit set. Therefore, if part of the afternoon were changed into a functional night, by daily covering tomatoes from 3:00 P.M. (war time) on, no loss of photosynthesis would occur, and an optimal night temperature would exist for a few hours.

To find out whether by these means tomato plants, growing outside, could be made to produce fruits out of season, some were planted in the field in the middle of November, 1943. Each afternoon at 3:00 P.M. half

[1] F. W. Went, *Am. Jour. Bot.*, 31: 135–150, 1944.
[2] F. W. Went, *Am. Jour. Bot.*, 31: in press.