with normal kidneys and in comparison with the control kidney of the unilaterally operated dogs, showed a marked decrease in the concentration of cytochrome c and also a diminution in the activities of the cytochrome oxidase and succinic dehydrogenase systems. The rate of oxidation of pyruvate and of l(+)-glutamate as well as the rate of synthesis of carbohydrates from these substances was greatly decreased. The rate of formation of ammonia from the oxidative deamination of l(+)-glutamate was considerably reduced in these kidneys. The protein-bound phosphorus content was lower in the kidneys whose renal artery has been partially constricted or which were wrapped in Cellophane or silk.

When the renal artery of only one kidney was partially constricted or one kidney wrapped in Cellophane or silk, the control kidney, examined one to six months after operation, showed an increase in cytochrome c concentration, cytochrome oxidase, succinic dehydrogenase activities per gram of tissue of from 15 to 35 per cent. over and above the average of normal kidneys. Also after unilateral nephrectomy the concentration of cytochrome c, of flavin-adenine dinucleotide and of protein-bound phosphorus was about 20 to 40 per cent. higher in the remaining kidney.

Solutions of renin prepared according to the method of Helmer and Page[5] inhibited the activity of the cytochrome oxidase, the succinic dehydrogenase, the l-amino acid oxidase and the amine oxidase systems. The degree of inhibition ranged from 10 to 80 per cent., varying with the individual enzyme and with the amount of renin added. It must be noted, however, that pure renin has not yet been isolated and that the renin solutions used may contain other factors responsible for the inhibitory effect demonstrated.

Preparations of kidney tissue obtained from hypertensive dogs showed similar inhibitory effects. Heating at a 100° C for 5 minutes destroyed a great part of the inhibitory activity of these tissue preparations.

It has been reported that in experimental renal hypertension, the concentration of renin is increased in the kidney itself[6,7] and in its venous blood.[8,9]

Since renin is a proteolytic enzyme, it may, if it has access to the respiratory enzymes mentioned above, be responsible, directly or indirectly, for at least part of the decrease in the activities of the enzymes reported in this paper and in a previous study.[10]

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THE MILK AGENT IN SPONTANEOUS MAMMARY CARCINOMA[1, 2]

PREVIOUS workers have shown that the milk agent for spontaneous mouse mammary carcinoma[3] is present in milk, in lactating mammary glands and in spontaneous or transplant tumor tissue, and that it can be at least partially sedimented or is associated with particles which are sedimented, in centrifugal fields of 20,000,[4] 60,000[5] and 110,000[6] times gravity.

In the experiments reported here spontaneous or transplant tumor tissue was used. The material was homogenized with distilled water, buffers or saline solution and then either was lyophilized or spun in a centrifuge and the supernatant treated in various ways.

The test animals were hybrids between C57 black mothers and A strain fathers or the back-cross mice from these hybrids to A males. In addition a few fostered C3H or A strain mice were used. The animals were between 4 and 10 weeks of age when they were injected with the various fractions mentioned below.

Results: One experiment, dealing with the stability of the milk agent toward heat, made use of the water extract of transplant tumor tissue. The supernatant was divided into 5 aliquot portions and these were kept at 4°, 37°, 60° or 90° C for 1 hour or at 24° C for 2 hours. All portions were brought to room temperature and an amount equivalent to 1 gram of original tissue was injected. The results are shown in Table 1. All animals in this experiment are now dead.

In another experiment frozen tumor tissue was extracted with saline solution and spun at 15,000 g for 30 minutes. Part of the supernatant was extracted with petroleum ether and gave rise to 5 tumors in 8 animals. The remainder of the supernatant was centrifuged at 50,000 g for 1 hour. The sediments at 15,000 and 50,000 g, the supernatant at 50,000 g and the Berkefeld N filtrate of the original saline extract gave rise to 1 or 2 tumors each out of 10 animals. An attempt to fractionate this same saline extract by

[1] From the Divisions of Physiological Chemistry and Cancer Biology of the Department of Physiology, University of Minnesota. Preliminary report.

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TABLE 1

Treatment	No. of mice	No. of tumors	Per cent. with tumors	Average tumor age, mo.	Average age of non-cancerous mice at death, mo.
4° 1 hour	18	9	50	16	18
24° 2 hours	17	6	35	15	18
37° 1 hour	19	4	21	16	19
60° 1 hour	18	0	0	..	20
90° 1 hour	18	0	0	..	20

$(NH_4)_2SO_4$ precipitation resulted in various fractions which gave rise to only 1 tumor out of 50 animals. This must be interpreted as meaning that the salt *per se* or the manipulations involved in the fractionation brought about an inactivation of the agent, or possibly that the petroleum ether removed some inhibitor.

Experiments in which phosphate buffer extracts of tumor material were incubated at room temperature at various pH values throw some light on the pH stability of the agent. Tumors have been noted from extracts kept at pH 5.0, 5.5, 6.3, 6.9, 8.7 and 10.2 for 1 or 2 hours. No tumors were noted after treatment at pH 4.5 for 2 hours, but such a negative finding will require confirmation. In general, the material soluble after incubation gave rise to more tumors than did the precipitates, even at pH 5.0, but these differences probably are not significant.

Another experiment which is not yet complete deals with acetone or petroleum ether extraction of lyophilized tumor material. So far 2 of 13 animals given ether-extracted material and 4 of 15 animals given acetone-extracted material have developed tumors. No tumors have developed from the acetone extracts, but in one group given an ether extract 2 of 10 animals have had tumors. This last material, however, differed from the other acetone and ether extracts in that it showed a granular precipitate as well as an oil when the solvent was removed.

Two recent experiments, both incomplete at the present time, deal with an attempt to precipitate the agent with the basic protein, salmine. The animals used in these experiments were all between 4 and 6 weeks of age. The starting material in both experiments was spontaneous tumor tissue, which in one case was used fresh and in the other was kept in the frozen state for several weeks. The tissue was extracted with saline or distilled water and the supernatants collected after centrifuging at about 2,000 g for 20 to 30 minutes. In one case the supernatant was adjusted to pH 5.5 and the soluble fraction treated with various concentrations of salmine. In the other case the original supernatant, which had a pH of 6.8, was treated with salmine. Tumors have already appeared from all fractions tested in both experiments with the exception of one of the sediments occurring after salmine treatment. The combined results of the two experiments are summarized in Table 2.

TABLE 2

Material	No. of mice	No. of tumors	Per cent. with tumors	No. still living	Average tumor age, mo.
Orig. S.N.	17	8	47	4	12
S.N. pH 5.5	10	4	40	2	13
Sed. pH 5.5	10	3	30	4	13
Salmine S.N.	38	17	45	11	12
Salmine Sed.	38	4	10	19	13

S.N. denotes supernatant; Sed. denotes sediment.

Perhaps the most important conclusion to be drawn from this experiment is that the one gram equivalent of tumor tissue, which all mice received, is much more than the minimal effective amount since tumors are developing from all fractions tested despite careful washing of all sediments. Experiments are now in progress to determine the minimal effective amount.

Summary: These experiments appear to show that the milk agent is destroyed at temperatures of 60° C and above, that it is stable at pH values between 5.0 and 10.2 but not at pH 4.5, that it is not inactivated by petroleum ether or acetone and not appreciably soluble in these solvents, and that it is partially, though perhaps only slightly, precipitated by salmine at pH values of 5.5 and 6.8.

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INHIBITION OF THE BACTERIOSTATIC ACTION OF MALACHITE GREEN BY ITS LEUCOBASES

THE bacteriostatic action of triphenylmethane dyes for Gram positive microorganisms is a well-known fact (see Churchman[1]). On the other hand, it is also generally acknowledged that the leucobases of these dyes are not bacteriostatic.

We carried out bacteriological assays with different leuco-derivatives of malachite green, such as leucobases, carbinol bases, bisulphite and hydrosulphite derivatives (leucosulphonic and leucosulphinic acids) and we found that:

(a) Carbinol bases, bisulphite and hydrosulphite derivatives possessed a bacteriostatic activity, no less, than the original malachite green dye (chloride or oxalate), *i.e.*, they were active until dilutions of 10^{-6} inclusively.

(b) The leucobases of malachite green had not only no bacteriostatic action in a dilution of 10^{-4}, but it

[1] Churchman, in Jordan-Falk's "The Newer Knowledge of Bacteriology and Immunology." Chicago. 1928.