interval between excitation of the right and left ventricles was found by Lewis to be only 0.01 seconds. As we found it, the interval was greater; this may be attributed to the fact that we measure the interval between the beginning of the ejection from the two chambers. We know that in the human heart the isometric contraction of the right ventricle lasts 0.01 sec. (Richards and coworkers[11]) but we do not yet know the duration of the isometric contraction of the left. It is likely that the higher pressure existing in the aorta causes a slightly longer duration of the period of tension of the left ventricle, thereby exaggerating the delay already existing.

Our observations on the pulsations of the auricles indicate that, here too, there is a brief interval, the right auricle contracting before the left. Again our observation confirms known physiological facts. In the dog, the sino-auricular impulse reaches the left atrial appendage 0.015 seconds later than the right (Lewis,[10] Wiggers[12]). It is logical to assume that the interval is longer in the human heart owing to the greater length and difference in length of the muscular bundles leading from the sino-auricular node to either auricle respectively.

The constancy of our findings is in contrast with the variability of results of previous studies.[1,2] This can be explained by the fact that those studies were performed partly with indirect measurements[1] or with a different technique.[2]

Summary. The temporal relation between the contractions of the left and right heart chambers was studied by means of fluorocardiography and simultaneous recording of 2 pulse tracings on one strip. The study was performed on 8 normal subjects and included the observation of the pulsations of the aorta and the pulmonary artery, both auricles and both ventricles. In all observations the contraction of the right auricle preceded that of the left auricle and the contraction of the right ventricle that of the left. The delay of action of the left chambers was found to be between 25 and 30 milliseconds.

11 Richards, D. W., Cournand, A., Motley, H. L., Dresdale, D. T., and Ferrer, M. I., *Trans. Assn. Am. Phys.*, 1947, in press.

12 Wiggers, C. J., *Physiology in Health and Disease*, Philadelphia, Lea and Febiger, 1944.

16117

The Effect of a *Sporosarcina ureae* Preparation on Tumor Cells *in vitro*.*

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The report that cell division in sea urchin eggs was inhibited by penicillin[1] was the incentive for these experiments designed to test the effect of microbial products on tumor cells. Cornman[2] and Lewis[3] found tumor cells in tissue culture damaged or killed by the addition of suitable concentrations of penicillin, though Lewis believed the selective carcinoclastic effect was due to impurities in the preparations.

In experiments not otherwise reported, we injected penicillin into Webster strain mice bearing spontaneous mammary carcinomata. Damage to the tumor in the form of hemorrhage, necrosis, and pyknosis was apparent but only after 50-100,000 units of penicillin

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1 Henry, R. J., and Henry, M. D., *J. Gen. Physiol.*, 1945, **28**, 405.

2 Cornman, I., *J. Gen. Physiol.*, 1944, **28**, 113.

3 Lewis, M. R., *Science*, 1944, **100**, 314.

were administered daily for more than a week.

A search for other carcinoclastic microbial products was then undertaken. For a survey of a large number of micro-organisms, *in vivo* test is too slow and interpretation is likely to be uncertain. Accordingly the test chosen was the histological condition of surviving tissue slices, as used in metabolism studies, after incubation for 24 hours with the bacterial preparation under investigation. A slice of the liver of the same animal was used as an indicator of the selectivity of the preparation for tumor or normal tissue. Penicillin, it was found, could be used to exclude extraneous micro-organisms.

Materials and Methods. Webster strain mice bearing spontaneous carcinomata, and pigmented rabbits carrying the transplantable Brown-Pearce carcinoma supplied the tumor and liver slices used in these experiments. Tissues which showed extensive necrosis or were otherwise unusual were discarded.

Mice were killed by a blow on the head and rabbits by injecting air into a marginal vein. The tumor and liver were removed, rinsed in physiological saline solution, and the tissues sliced into sections not more than 0.3 mm thick, either with a straight razor or a Stadie[4] microtome. Mouse liver was generally cut by hand, while mouse tumor and rabbit liver and tumor were more conveniently cut on the microtome. The slices were rinsed through 3 baths of physiological saline solution before being placed in 20 ml beakers for incubation. Each beaker contained 4 ml of the medium, a slice of tumor, and as a control tissue, a slice of the autologous liver.

All procedures were carried out as aseptically as possible.

Previous tests showed that tissues incubated in serum under conditions later described preserved a more nearly normal gross and microscopic aspect than they did when incubated in balanced salt solution. Heterologous serum appeared to be as favorable as the homologous serum, consequently the basic medium consisted of rabbit serum obtained from blood collected by aseptic cardiac puncture to which 100 mg of dextrose and 100,000 units of penicillin (Squibb) were added per 100 ml of serum before use. The usual procedure employed 3.5 ml of serum containing sugar and penicillin plus 0.5 ml of the culture or preparation being tested.

A shaker-incubator[5] equipped to hold thirty 20 ml beakers allowed the agitation to be carried out at any desired speed, temperature, and under any preferred water-saturated gas mixture. Tissues in these experiments were incubated for 24 hours at 37°C under 95% oxygen and 5% carbon dioxide and shaken at 80 cycles per minute.

All preparations were run in duplicate, and with each set of experimental beakers a pair of beakers containing only serum and the tissue slices were incubated as control. In experiments not involving the use of penicillin in all beakers, an additional control pair without penicillin was run.

Specimens of tumor and liver were routinely fixed in Bouin's or Carnoy's fluid at the beginning of each experiment, and at the conclusion, all tissues were immediately fixed in preparation for microscopic examination.

The slices were sectioned at 8 μ in a plane parallel to the original slicing to give large area for examination. Orientation was assured by sandwiching the slices between sheets of agar gel prior to fixation.[6] Hematoxylon-eosin was the routine stain.

The basic medium for growing the bacteria was as follows:

Yeast extract (Difco)	3 g
Peptone (Difco)	3 "
Dextrose	5 "
Tapwater	1 L

Adjusted to pH 8.0 with NaOH prior to autoclaving.

Most of the organisms tested grew well in this medium at room temperature. For the culture of *Sporosarcina ureae* and other urea decomposing bacteria 20 g urea was added to the above medium.

Results. In a general survey of micro-organisms whose products might selectively

[4] Stadie, W. C., and Riggs, B. C., *J. Biol. Chem.*, 1944, **154**, 687.

[5] Dubnoff, J. W., in preparation.

[6] Cohen, A. L., in preparation.

damage tumor tissue, the variety of growth habits made it necessary to modify the conditions of testing them. Thus bacteria which could grow rapidly in serum at 37°C were added as small inocula; those which might grow to some extent but not sufficiently to swamp the tissues were added either as loopfuls of rich culture to beakers containing 4 ml of serum minus penicillin or as 0.5 ml concentrates of rich cultures to 3.5 ml of serum containing penicillin; while those organisms which could not grow under the conditions described were added as 0.5 ml concentrates to each beaker containing 3.5 ml serum with penicillin added to suppress the growth of contaminants.

Some bacteria damaged both liver and tumor cells, the severity of injury ranging from slight eosinophila and paling of the nuclei to complete disintegration. These were: *Pseudomonas caryocyanea, P. putida, P. sp., Serratia marcescens, Escherichia coli communis, E. aurescens, Shigella sonnei* and *Sarcina sp.*

The majority of micro-organisms tested exhibited no perceptible effect upon either the tumor or liver. In this category are *Pseudomonas indoloxidans, P. ovalis, P. omnivorans, P. acidovorans, P. pyocyanea, P. tumefaciens, Vibrio metchnikovii, Rhodospirillum rubrum, Escherichia coli* (2 strains), *E. coli communior* (compare with *E. coli communis* of preceding list) *E. acidi-lactici, Aerobacter aerogenes, A. cloacae, Alcaligenes fecalis, Salmonella pullorum, Bacterium alboflavus, Proteus vulgaris, Kurthia zopfii, Staph. citreus, Strep. anhemolyticus, Strep. sp., Micrococcus ureae, M. cinnabareus, Sarcina sp., Bacillus brevis, Aerobacillus polymyxa, Corynebacterium creatinovorans, Mycobacterium phlei, M. salmonicolor, M. sp., Actinomyces coelicolor, Penicillium notatum.*

In addition to these organisms 3 trypanosomes, *Trypanosomum brasiliensis, T. lewisii,* and *Schizotrypanosomum cruzii* were tested because of Roskin's report[7] of their use in producing tumor regression. They were ineffective in our test.

A perceptible specific carcinoclastic effect was produced by preparations of the following organisms: Bacterium I (an unidentified, slender, motile, non-sporulating rod isolated from contaminated serum), *Spirillum virginianum, Phaeomonas varians, Urobacillus pasteurianum,* and *Sporosarcina ureae.* Of these 5 organisms *Sporosarcina ureae* had the most pronounced differential destructive effect upon tumor tissues. It was selected, therefore, for further study.

After incubation with *Sporosarcina ureae* a slice of spontaneous carcinoma of the Webster mouse underwent the following degenerative changes: The nuclei became pyknotic, the cell membranes shrank and the characteristic acinar structure was largely obliterated. The liver slice cells appeared to be undamaged.

In the first experiments, *Sporosarcina ureae* was grown in 50 ml Erlenmeyer flasks containing 10 ml of culture medium at room temperature. The cultures were harvested at the peak of growth and 0.5 ml of culture was added to each beaker containing 3.5 ml of serum with mouse liver and tumor slices prepared as previously described. Experiments were made with and without the use of penicillin in the concentration of 1000 units per ml. No effect on the action of the *S. ureae* culture being observed, all further experiments were done with penicillin in the medium to safeguard tissues and serum against the growth of contaminants.

Because of the high concentration of ammonia in the *Sporosarcina ureae* cultures addition of 0.5 ml to 3.5 ml of serum gave a pH of 9.5. It was possible, then, that the specific toxicity of the *Sporosarcina ureae* culture to tumor cells resided in either the ammonia present in high concentration or the high pH induced rather than in a more specific toxic substance. To test these possibilities tumor and liver slices were incubated in the serum medium to which either ammonium hydroxide or sodium hydroxide were added to give a pH of 9.5 or higher. No damaging effect of either ammonium or hydroxyl ion at these concentrations was observed on either the tumor or liver cells. Nor did removal of the ammonia from various *Sporosarcina ureae* preparations lessen their

[7] Roskin, G., *Cancer Research,* 1946, **6**, 363.

selective carcinoclastic effects.

In an attempt to enhance the effect *Sporosarcina ureae* was grown under the following different conditions:

A. Two-liter flasks containing 500 ml of culture fluid inoculated with *S. ureae* were incubated at 30°C for 48 hours with constant agitation. These heavy cultures harvested at the peak of growth produced a slighter effect than those grown under less favorable cultural conditions. Further experiments indicated that the carcinoclastic effect was greater with cultures which had passed the peak of growth.

B. A 500 ml culture was prepared, agitated at 30°C for 48 hours, and allowed to remain undisturbed at room temperature (ca. 22°C). After 48 hours a sample (BI) was lyophilized, and the remainder (BII) was lyophilized after 120 hours at room temperature. When reconstituted with distilled water sample BI showed little or no carcinoclastic effect, while tumor tissue exposed to sample BII was totally destroyed, the nuclei densely pyknotic, cell boundaries extinguished, and the acinar structure collapsed. There was no perceptible effect upon the liver.

In studies with whole culture, washed bacteria and cell-free medium, it was regularly observed that most pronounced carcinoclasis was obtained either with the washed concentrated cells or with whole old culture.

C. Plates were made of the yeast extract urea medium solidified with a 2% agar, heavily seeded with *S. ureae* and incubated at 30°C for 48 hours. The bacteria were washed off with distilled water, the washings collected and lyophilized. The bacteria from four 200 mm diameter petri dishes were resuspended in 20 ml distilled water after lyophilization, a few drops of methylene blue added as redox indicator, and the suspension divided into four 5 ml lots incubated 18 hours under the following conditions:

C I. Anaerobic at 55°C Methylene blue incompletely reduced.

C II. Anaerobic at 36°C Methylene blue incompletely reduced.

C III. Room temperature under toluene. Methylene blue incompletely reduced.

C IV. 30°C under toluene. Methylene blue reduced in one-half hour. Toluene removed from C III and C IV by vacuum drying, residue reconstituted to original volume.

Preparation C IV, that autolysed under toluene at 30°C, produced complete disintegration of the tumor tissue and did not damage the liver as far as could be seen from stained preparations. Next in order of effectiveness were preparations C I and C II. C III had little effect.

Rabbit Brown-Pearce carcinoma was tested in a similar manner as the Webster strain mice carcinomata (above) and similar results were obtained.

The factor in *Sporosarcina ureae* toxic to tumor and not to liver cells appears to be contained within the bacteria and increases in the medium as these are aged. The evidence for this conclusion is the greater effectiveness of old cultures, the complete and selective destruction of tumor tissue by the bacteria autolyzed at 30°, and the ineffectiveness of the cell-free culture medium in which the bacteria had grown.

Discussion. As far as we are aware, the test method used in this study has not been employed hitherto in the study of or search for substances selectively toxic to tumor cells. It holds out the following advantages: it is rapid, economical, the one animal, even the one tissue, supplies control and experimental specimens, and indirect (immunological and vascular) effects of the test substances are inoperative.

Kidd[8] used a somewhat analogous approach. He found that certain tumor cells after treatment with culture filtrates of *Aspergillus fumigatus* failed to grow after implantation, although there was no visible injury in the tumor cells.

Summary. The incubation of mouse spontaneous carcinoma, rabbit Brown-Pearce carcinoma, and their autologous liver slices with bacterial cultures showed in general that cells of both liver and tumor were uninjured or injured equally. Cultures of *Sporosarcina ureae* damaged tumor cells and left liver cells histologically unaffected. Evidence was obtained that the selective carcinoclastic factor

[8] Kidd, J. G., *Science*, 1947, **105**, 511.

of *S. ureae* cultures was contained within the cells and released on autolysis.

We are indebted to Dr. C. B. van Niel of the Hopkins Marine Station for free access to the culture collection, to Colonel Max Levine for cultures of several of the Enterobacteriaceae, and to Dr. William Negherbon for cultures of trypanosomes and detailed directions for their maintenance.

16118

Liver Injury in the Dog Following Use of 2-3-Dithiopropinol.* (BAL).

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Fifteen adult dogs furnish the basis for the observations which are to follow. The animals were kept in metabolism cages and given a diet of Purina Dog Chow. No restriction as to the amount was made in the diet or in the water intake. The dithiol was given intramuscularly at 9 a. m. and 9 p. m. on 4 successive days. The dogs were divided into 3 groups. The animals of Group I were given 5 mg per kilo of the dithiol per dose, those of Group II, 15 mg and the members of Group III, 30 mg per kilo. Prior to the commencement of the injections liver function studies were undertaken by the use of bromsulfalein according to the technique devised by Rosenthal and White.[1] For the normal animals and those of Group I, such observations have been satisfactory in that all of the dye was removed from the plasma within half an hour. In the animals of Group II and Group III receiving respectively 15 and 30 mg of dithiol per kilo the rate of removal was invariably prolonged beyond the half-hour period. The percentage removal however during this and subsequent periods was extremely variable. The evidence for the development of a liver injury from the dithiol is not confined to such variable functional observations but has been ascertained by obtaining biopsy material from the livers and by the study of such tissue in those animals that came to autopsy. The tissue was stained for lipoid material with Scharlach R and also with hematoxylin and eosin. A study of such material permits the following conclusions:

1. In the animals of Group I which received 5 mg of 2-3-dithiopropinol per kilo it was difficult if not impossible to ascertain by such a micro-chemical method whether or not there was any actual increase in lipoid material in the hepatic epithelium over that which can be frequently observed in such tissue designated as normal. In such animals lipoid material appears in the liver in the form of dust-like and larger particles or as minute droplets. This material is more noticeable in the periphery of the lobules following the use of a dithiol.

2. In the animals of Groups II and III which received either 15 or 30 mg of the dithiol per kilo, there develops a marked increase in stainable lipoid in the liver lobules in the form of larger or smaller droplets which may obscure the nuclei of such cells and replace the greater portion of the cell cytoplasm. Such a development is more uniform and more marked in the periphery than in the central portions of the lobules. In general as this area of the lobule is reached the lipoid material is not only reduced in amount but makes its appearance as small discrete droplets. This order of hepatic cell injury is more severe and develops earlier in the animals of Group III that were given 30 mg of the dithiol per kilo. In one animal of this group there developed by the fifth day of the experiment an extensive liver necrosis. The animal became comatose with

* 2,3-dithiopropanol (BAL), 10%, benzyl benzoate 20% in peanut oil.

1 Rosenthal, S. M., and White, E. C., *J. A. M. A.*, 1925, **84**, 1112.