A STUDY OF SOME DIAGNOSTIC REACTIONS FOR MALIGNANT TUMORS

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Under the auspices of the Huntington Fund for Cancer Research I have investigated some of the serum-diagnostic tests for malignant tumor, chiefly those of Freund and Kaminer and of von Dungern, and it is the purpose of this communication to report the results of that study.

The serological diagnosis of disease is made with the aid of reactions that are designated as "bio-chemical" reactions because of the fact that the chemical constitution of the substances taking part in those reactions is for the greater part unknown. The first serological test applied to the diagnosis of disease (the Widal reaction) depended upon the interaction of true antigenic substances with their specific antibodies, in the phenomenon of agglutination. As other expressions of specific immunity reactions were discovered (specific complement deviation, meiotstagine reaction, anaphylactic shock) attempts were made to use these also in the diagnosis of disease, and as a result of such efforts a perfect imitation of specific complement deviation was discovered in the non-specific Wassermann reaction, a reaction that is produced by the interaction of lipoid substances lacking true antigenic properties, and a serum constituent that is not a true antibody.

In the search for a diagnostic serological test for malignant tumors, the investigations have generally followed the lead of the specific as well as of the non-specific immunity reactions, but some tests have been devised that are not based on any of these reactions.
The experimental basis of the search for a specific immunity reaction in cancer is found chiefly in the studies upon immunization against normal tissues. These have demonstrated antibody production against tissues derived from foreign species, against the cells of animals of the same species (1) and against the cells of certain of the individual’s own organs (2) (kidney, pancreas and spleen). Ample ground seemed to be furnished by the results just cited for the assumption that the immunity often observed against the inoculation of homogenic transplantable tumors was due to the influence of cytotoxic antibodies, yet such antibodies have never been found. Moreover, the methods of specific precipitation and complement fixation, also, have generally (3) failed to reveal the development of antibodies in animals bearing true transplantable tumors.

In human cancer, specific antibodies have been sought with the use of the method of complement fixation, the anaphylactic reaction, and the meiostagmine reaction. The method first mentioned has failed completely in a number of hands. Ludke (4), however, believed that he could show, with the serum of two cases of human carcinoma, a slight specific complement deviation, and Simon and Thomas (5), using the hemolytic system, hen corpuscles, anti-hen corpuscle—rabbit’s serum and guinea pig complement, obtained complement deviation in 24 out of 37 cases of malignant tumor, by uniting the patients’ sera with a quantity of cancer extract that by itself was not anticomplementary. They obtained no positive reaction in 50 cases of normal or otherwise diseased individuals.

Ranzi (6) injected 10 cc. of a paper-filtered cancer extract into two cancer patients and after one week examined the two sera with the method of complement deviation. His results were entirely negative. Similar experiments were carried out by Lebredo and Coca (7), all of which resulted negatively.

The anaphylactic reaction has been used in both its local and its general expression. von Dungern and Gorowitz (8) reported a specific local hypersensitiveness in cancerous individuals to extracts of malignant tumors. Their observations have not been confirmed. Pfeiffer and Finsterer (9) employed the method
of passive sensitization to demonstrate specific immune bodies in human individuals suffering from cancer. Three guinea pigs received intraperitoneal injections of 4 cc. of the serum of human cancer patients. On the following day, each animal received intraperitoneal injections of 4 cc. of the expressed juice of human cancer. All three of these animals exhibited symptoms of hypersensitiveness from which they recovered. Five control animals, that previously had been treated with normal human serum, and two others not previously injected, showed no symptoms upon receiving similar inoculations of the cancer juice. Ranzi (10), in a series of nine cancer cases and fifteen controls, failed entirely to confirm the results of Pfeiffer and Finsterer. Ranzi's test injection was made intravenously and not intraperitoneally, as prescribed by Pfeiffer and Finsterer. Philosophow (11), in two cases, also, was unable to confirm the experiments of Pfeiffer and Finsterer. Later, Pfeiffer (12) published a larger series of experiments by which he upholds the results obtained with Finsterer. According to these experiments, tumor-specific antibodies are demonstrable in the blood of individuals suffering from carcinoma but not of those afflicted with sarcoma or non-malignant tumors. The usual criteria of hypersensitiveness—convulsions or death—were not used by Pfeiffer and Finsterer; they employed the single criterion of temperature drop. This phenomenon was first employed by Pfeiffer (13) in the detection of the lesser degrees of anaphylactic reaction, and it was later claimed by Mita (14) that with the use of a certain formula the degree of shock could be exactly calculated by noting the temperature curve over a period of several hours after the toxic injection.

The writer was discouraged from undertaking a further study of the Pfeiffer reaction in cancer on account of some unpublished experiences of Dr. Richard Weil and himself. It was found, first, that the rectal temperature of normal guinea-pigs varies greatly according to the depth to which the thermometer is inserted; and, secondly, that in many animals a marked lowering of the rectal temperature is caused by merely strapping the animal on the operation board.
In the class of non-specific reactions are to be placed the Freund-Kaminer reaction, and probably also, the complement fixation test of von Dungern, notwithstanding the fact that the latter reaction is carried out according to principles drawn originally from the interaction of specifically related bodies.

The Freund-Kaminer test for malignant tumor is based upon the observation of the authors that the isolated cells of carcinoma are dissolved by the serum of non-cancerous individuals, whereas this property is wanting in the sera of the cancer patients. The technic of the test is as follows:

**Preparation of the cancer cell emulsion**

The non-necrotic portions of metastatic liver carcinoma are cut into small cubes and allowed to stand in the ice-box over night in 0.6 per cent NaCl solution containing 1 per cent of acid sodium phosphate. The tissue is then crushed by hand in a thin, fine-meshed towel under the acid sodium phosphate mixture, in such a manner that the isolated cells and small groups of cells are forced through the towel into the fluid outside. The cells are now washed ten times with 0.6 per cent saline solution with the use of the centrifuge (at slow speed for two or three minutes) to remove the liver cell débris, and at the end of this process the suspension is centrifuged for one-quarter minute at lowest speed, and the sediment, containing the larger tissue fragments and groups of cells that have not been separated, is discarded. The final suspension should contain only isolated cells and no cell fragments; the preparation of the cell emulsion is conducted without any precautions directed to the exclusion of bacteria. The cells in the resulting suspension are counted, and the suspension is diluted to a point at which 1 drop in 20 will give the desired number of cells per field of 16 small squares in the Thoma-Zeiss blood-counting chamber. This number has been placed by Freund and Kaminer at between 15 and 25. Finally, the cell suspension is mixed with a tenth volume of a concentrated (5 per cent or 10 per cent) solution of sodium fluoride. According to Freund the fluoride solution should be slightly alkaline to litmus. The cell suspension is kept in the ice-box.
The test

Twenty drops (about 1 cc.) of the freshly obtained patient’s serum are mixed in a clean narrow glass tube with one drop of the (shaken) cell suspension and two drops of the concentrated sodium fluoride solution. The number of cells per 16 small squares of the Thoma-Zeiss blood-counting chamber is determined in a drop of the mixture, and the tube, after being tightly closed with a stopper, is placed in the incubator at 37°C. for twenty-four hours. A second count of the cells is then made in order to see whether the number of cells has diminished. It is not required that the glass tubes or pipettes be sterile.

With ether Freund and Kaminer were able to extract, from normal sera, fatty acids which destroyed the isolated cancer cells. As these acids, according to Freund and Kaminer, are lacking in the serum of cancer patients, they are believed to be the active carcinolytic substances in normal sera.

The cell dissolving property of normal serum is destroyed by heat (56°C. for one-half hour). It may be preserved in serum kept in the ice box for about one week, but it is lost within two days at room temperature.

Cancer sera possess the power of inhibiting the carcinolytic property of normal sera. This inhibiting power is believed by Freund and Kaminer to be exercised by the nucleo-euglobulins which, in cancer serum, possess chemical characters different from those of the nucleo-euglobulins of normal serum. This difference is particularly indicated by a different result with the Molisch carbohydrate reaction.

The observations of Freund and Kaminer were confirmed by Ranzi and Amiradzibi (15), by Arzt (16), by Stammler (17), by Kraus and Graff (18), who found further that the blood of the umbilical cord behaves toward the cancer cells as does the blood of cancer patients, in that it lacks carcinolytic properties; by Kraus, von Graff, and Ranzi (19), by von Monakow (20), who, however, observed over 50 per cent destruction of the cancer cells with only 16 out of 52 normal sera; and by Arzt and Kerl (21).

My own experience in the Freund-Kaminer phenomenon began in the chemical laboratory of the Rudolf-Stiftung under the
direction of Professor Freund. There were placed at my disposal 0.6 per cent sodium chlorid solution (not sterile) 10 per cent sodium fluorid solution (Merck's Fluornatrium puriss) and eight different emulsions of the cells of secondary carcinoma of the liver. Examination of these cell emulsions showed three of them to contain cells of uniform size with almost no fragments, the presence of which might disturb the counting of the cells. These three emulsions were used in the subsequent experiments.

The cell emulsions were of such a density that when one or two drops were mixed with 1 cc. (about 20 drops) of serum and two drops of the sodium fluorid solution, and a small drop of the mixture was placed in the Thoma-Zeiss blood counting chamber, the average number of cells in a field of 16 small squares lay usually between 15 and 25.

The first experiment was carried out with fresh serum from a case of carcinoma of the breast and from a case of advanced arteriosclerosis (non-cancerous), each of which was tested with two of the cell emulsions. The results were as follows:

**Cell emulsion A**

<table>
<thead>
<tr>
<th></th>
<th>Cancer serum</th>
<th>Arteriosclerosis serum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At once</strong></td>
<td>9, 14, 5, 11, 11, 16, 12, 14, 7 (av. 11)</td>
<td>15, 13, 12, 9, 16, 14 (av. 13)</td>
</tr>
<tr>
<td><strong>After 24 hours</strong></td>
<td>11, 11, 9, 10, 7, 10, 15, 5 (av. 10)</td>
<td>8, 5, 4, 5, 6, 5, 5 (av. 5.5)</td>
</tr>
</tbody>
</table>

**Cell emulsion B**

<table>
<thead>
<tr>
<th></th>
<th>Cancer serum</th>
<th>Arteriosclerosis serum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At once</strong></td>
<td>22, 20, 25, 22, 21 (av. 22)</td>
<td>18, 23, 29, 22, 24 (av. 23)</td>
</tr>
<tr>
<td><strong>At once</strong></td>
<td>23, 20, 27, 23, 23 (av. 23)</td>
<td>6, 5, 4, 9, 4 (av. 5.6)</td>
</tr>
</tbody>
</table>

1 The 10 per cent solution of sodium fluoride was prepared according to Freund and Kaminer by shaking 10 gr. of the substance with 100 cc. of water and filtering the mixture. The filtrate did not contain 10 per cent nor even 5 per cent of sodium fluoride, since the saturated aqueous solution of this substance contains less than 5 per cent of it by weight. In fact, the solids in the filtrate were found, by weighing the dry residue from 1.0 cc. of the filtrate, to amount to 4.5 per cent, which shows the solution to have been not quite saturated.

2 Between 5 and 10 fields were counted and the average of these was taken.
It is seen that, when mixed with the non-cancerous serum, the cells of both emulsions were markedly diminished in number after twenty-four hours at 37°C., whereas the cancer serum left the number of cells, in both instances, practically unaltered.

The next series of tests was carried out with the object of studying the nature of the cytolytic agent in normal sera. For these experiments, fresh normal human sera and horse serum were used, the other reagents being the same as in the first experiment. However, in these tests no cytolysis took place.

Bacteriological examination was made of the mixtures of this series before and after the twenty-four hour incubation, for the purpose of determining the antiseptic activity of the sodium fluorid. This examination was carried out by spreading one loop of each of the mixtures on the surface of nutrient agar and counting the number of colonies that had appeared at the end of forty-eight hours. The cultures taken before incubation showed either no growth or at most two colonies, whereas the cultures from all the mixtures taken after the twenty-four hours' incubation at 37°C. showed numerous colonies within twenty-four hours after the cultures were taken. The number of colonies that developed from the mixtures containing the sodium fluorid was, however, considerably less than those that developed from the mixtures in which sodium chlorid solution (0.6 per cent) was substituted for the sodium fluorid. This result indicates that the antiseptic action of the fluorid, as it is used by Freund and Kaminer, is slight. After numerous failures to obtain cytolysis with normal human sera, this phenomenon was again observed with a normal human serum and with normal horse serum. The human serum referred to was tested with two other normal sera and one cancer serum. In the mixtures with the latter three sera, the cancer cells were clumped and could not, therefore, be counted, but according to the gross appearance of the sediment as compared with the sediment in the salt solution control (containing no serum), only partial solution of the cells occurred with one of the other normal sera, while with the third normal serum and with the cancer serum no cytolysis could be detected. The first mentioned normal
human serum in this series caused almost complete solution of
the cancer cells, the count being:

At once........................................19, 22, 21, 23, 12, 27, 16 (av. 20)
After 24 hours 37°C........................................0, 0, 0, 1, 0, 0, 1

Bacteriological cultures from all the mixtures taken after the
twenty-four hours' incubation period, showed countless fine white
colonies. This result indicates that the cytolysis is neither hin-
dered nor produced by bacterial growth.

The horse serum just referred to was tested at the same time
with the sera of two normal rabbits, neither of which possessed
any cytolytic power. The horse serum dissolved about 70 per
cent of the cells of two different emulsions. In a single exper-
iment with this horse serum, an attempt was made to examine
the mechanism of the cancer cell cytolysis. The usual mixture
was prepared and allowed to stand at room temperature for
three hours, at which time no grossly apparent cytolysis had
taken place. The mixture was then centrifuged, and the sedi-
ment was mixed with 1 cc. of 0.8 per cent sodium chlorid solu-
tion and two drops of the saturated sodium fluorid solution
(mixture A), while the decanted supernatant fluid was mixed
with two drops of the cancer cell suspension (mixture B). The
cells in both of these mixtures were counted before and after the
incubation at 37°C. for twenty-four hours, with the following
results:

Mixture A
\[
\begin{align*}
\text{At once} & : 22, 22, 14, 26, 21, 25, 22 \text{ (av. 20)} \\
\text{After 24 hours} & : 24, 21, 18, 11, 8, 14, 14, 14 \text{ (av. 14)}
\end{align*}
\]

Mixture B
\[
\begin{align*}
\text{At once} & : 24, 21, 18, 18, 23, 17 \text{ (av. 20)} \\
\text{After 24 hours} & : 16, 15, 12, 25, 16, 12, 27 \text{ (av. 17)}
\end{align*}
\]

Control usual
\[
\begin{align*}
\text{Mixture} & : 16, 14, 18, 21, 25, 17, 25 \text{ (av. 19)} \\
\text{After 24 hours} & : 3, 4, 4, 7, 4, 4, 9 \text{ (av. 5)}
\end{align*}
\]

The distinct diminution (30 per cent) in the number of cells
in mixture A indicates that the cells at the end of three hours,
though still apparently intact, had already combined with some
of the cytolytic agent in the serum. It is possible that most of
the agent had been absorbed by the cells but was inhibited in
its action by unfavorable conditions of the saline menstruum.
At any rate, little, if any, of the cytolytic activity remained in the serum after its three-hour contact with the cells.

Further study in this direction was prevented by the fact that in none of the numerous subsequent tests has any cytolytic activity been observed in any fresh normal human or other serum.

The subsequent experiments were carried out in the cancer institute of the Eppendorferkrankenhaus in Hamburg, in the General Memorial Hospital in New York City, and in the New York Hospital. The cell suspensions used in Hamburg were prepared, according to the Freund-Kaminer prescription, from two metastatic carcinomas of the liver that were obtained from the pathological institute. Only non-cancerous sera were used, these being derived largely from syphilis and individuals suffering from tuberculosis and other diseases. The same sera were tested, also, with a cancer cell suspension brought in a thermos bottle from Vienna. The cell suspensions used in the General Memorial Hospital were prepared from a sarcoma and a cellular mammary carcinoma, both being fresh surgical material received from Dr. W. B. Coley. The sarcoma cell suspension could not be used, as the cells soon agglutinated into a gelatinous mass that could not be broken up by shaking.

A suspension of cells from a sarcoma of the testicle, obtained from operation at the New York Hospital, remained in suspension in the saline solution but became agglutinated when mixed with human serum. The other sources of cell material used at the New York Hospital were two metastatic liver carcinomas, one of which was received from the General Memorial Hospital through Dr. Ewing.

All of the more than 150 experiments in the three institutions resulted negatively, no cytolysis being observed in any instance with fresh normal human, dog, or horse serum.

It is evident from these experiences with the Freund-Kaminer phenomenon, that the cytolytic action of normal sera is dependent upon some factor as yet uncontrollable, and that that action, therefore, can not be made the basis of a differential test for malignant tumor.

The technic of von Dungern's complement-fixation test for
malignant neoplasms (22) has been modified in several respects by von Dungern since its first announcement.

The antigen extract consisted at first of an alcoholic extract of malignant tumors. For this preparation von Dungern substituted, in his second report, an acetone extract of human blood corpuscles, particularly those of individuals suffering from progressive paralysis. Hara (25) later used maltose and phenolphthalein as "antigen" in the test.

In the first experiments, the patient's serum was used unheated in a quantity of 0.05 cc. In his second report, von Dungern still used the same quantity of unheated serum, either as usual or with the addition of 0.2 cc. of n/50 NaOH. In his third publication, von Dungern recommended that the serum be heated for one-half hour at 54°C., after having been mixed with two parts of n/50 NaOH in about 0.6 per cent NaCl. Of this heated mixture, he used in series 0.6, 0.3, 0.15, and 0.075 cc. However, the tests were judged by the results with one quantity, 0.3 cc. of the mixture.

Von Dungern requires the use of sensitized ox blood corpuscles instead of the customary sheep's corpuscles, as the indicator of the cancer reaction, on the ground that they are less sensitive than the sheep's corpuscles in the desired capacity. Another departure from the usual Wassermann technic is the three hour fixation period at room temperature and the three hour incubation period for hemolysis, also at room temperature.

Petridis (24), working in von Dungern's laboratory, used the acetone extract of human blood corpuscles as "antigen," and followed the third method of von Dungern in the use of the patient's serum. Since the article of Petridis (24) there has been no published modification of the technic of the test, excepting, as has been said, the substitution, by Hara, of maltose and phenolphthalein for the "antigen" extract.

With the final technical form of the test von Dungern obtained nearly 91 per cent of positive reactions in malignant tumor, and 100 per cent of negative reactions in non-cancerous conditions, including syphilis. Halpern (25), in von Dungern's laboratory, obtained 89.8 per cent of positive reactions in malignant tumors,
with 92.8 per cent of negative reactions in non-cancerous conditions. Petridis (24) obtained 81.2 per cent of positive reactions in malignant tumors, and 84.2 per cent of negative reactions in non-cancerous conditions. Hara (26) reported about 84 per cent of positive reactions in malignant tumors, and over 97 per cent of negative reactions in non-cancerous conditions. Wolfsohn (27) obtained only 80 per cent of positive reactions in malignant tumors, and only 63 per cent of negative reactions in non-cancerous conditions. Wolfsohn found, further, that many luetic sera reacted positively with the cancer test as prescribed by von Dungern. A similar experience was reported by Edzard (28), who obtained only 70 per cent of positive reactions in malignant tumors, and only 65 per cent of negative reactions in non-cancerous individuals. Lindenschatt, on the contrary, obtained no positive cancer reactions with luetic sera.

Isabolinsky and Dichno (29) report unfavorable results with the test, and H. Sachs\textsuperscript{4} failed to confirm von Dungern's observations.

My first experiments with the cancer reaction of von Dungern were carried out in the Loomis Laboratory, with an acetone extract of normal human corpuscles and with sera of cancer patients obtained through the kindness of Dr. Richard Weil from the General Memorial Hospital. Thirty-six cancer sera and an equal number of non-cancerous sera were tested, and in no case was a fixation of complement obtained that could not be explained either as a summation effect, on account of the fact that the serum control, in which double the test amount of the serum alkali mixture was used, often caused a complete inhibition\textsuperscript{4} of the complementary action, or as an expression of a Wassermann reaction, which in a few instances was positive with the usual technic.

These experiments were continued in Hamburg in von Dungern's institute. There a second considerable series of cancerous and non-cancerous sera were tested with the blood extract

\textsuperscript{4} Personal communication.

\textsuperscript{4} Sometimes even the amount of the serum-alkali mixture used in the test was by itself slightly anticomplementary.
then in use in the routine examinations of the institute, and also with several other extracts of normal and syphilitic blood corpuscles. Here, again, my tests with the prescribed technic resulted negatively in every instance but one. Furthermore, all the routine tests of the institute that I saw during my stay in Hamburg resulted negatively, although many of these, also, were carried out with sera from cases of known malignant disease.

On two occasions, however, in which the prescribed technic was modified I obtained a clearly positive result, and in one of these instances the reaction appeared to be specific in a clinical sense. In the first instance, a single cancer serum and one Wassermann positive leutic serum were tested with the usual blood corpuscle extract and also with the lipoid (acetone insoluble) fraction of the same extract. The technic with both of these "antigen" preparations was that of the routine institute tests, and with both of the preparations the cancer serum reacted positively, whereas the leutic serum reacted negatively. A further series of seven cancer sera were tested according to von Dungern with the isolated human and pig's blood lipoids, the results being in every case negative.

In the second instance referred to above, the tests were performed, not with the serum but with the urine and with a solution of the alcohol precipitate of the urine of two individuals, one of whom was suffering from carcinoma, the serum of the other being strongly positive with the Wassermann test.

The patient's serum substitute was prepared as follows: 100 cc. of urine were mixed with 400 cc. of 96 per cent alcohol and the precipitate, after being washed once with 96 per cent alcohol, was taken up with distilled water up to 20 cc. The small part of the precipitate that remained was diluted one-fifth with physiological saline, and 0.025 cc. of this dilution was used in the tests in place of the patient's serum.

The urine in both cases was negative both to the usual tests for albumin and to the biuret reaction; the cancer urine was alkaline, the other was acid. In the concentrated solution of the alcohol-precipitate from the cancer urine, urobilin could be demonstrated spectroscopically, and its presence was further indi-
cated by a positive biuret reaction in the concentrated solution. The solution of the alcohol-precipitate from the non-cancerous (Wassermann positive) urine contained no demonstrable urobilin.

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<table>
<thead>
<tr>
<th>ACETONE EXTRACT OF GUINEA PIG'S HEART 1 PER CENT IN METHYL ALCOHOL</th>
<th>ACETONE EXTRACT OF WASHED HUMAN BLOOD CORPUSCLES (CASE OF PROGRESSIVE PARALYSIS) 1 PER CENT IN METHYL ALCOHOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cubic centimeters</td>
<td>Cubic centimeters</td>
</tr>
<tr>
<td>0.05       0.025 0.0125 0.00625</td>
<td>0.05 0.025 0.0125 0.00625</td>
</tr>
<tr>
<td>Kittelberger (carcinoma) urine, 0.025 cc.</td>
<td>Kittelberger (carcinoma) urine, 0.025 cc.</td>
</tr>
<tr>
<td>+ – – –</td>
<td>+ – – –</td>
</tr>
<tr>
<td>Alcoholic precipitate solution, 0.025 cc.</td>
<td>Alcoholic precipitate solution, 0.025 cc.</td>
</tr>
<tr>
<td>+ – – –</td>
<td>+ – – –</td>
</tr>
<tr>
<td>Same boiled, 0.025 cc.</td>
<td>Same boiled, 0.025 cc.</td>
</tr>
<tr>
<td>++ ++ +</td>
<td>++ ++ +</td>
</tr>
<tr>
<td>Schmitt (Wassermann positive) urine, 0.025 cc.</td>
<td>Schmitt (Wassermann positive) urine, 0.025 cc.</td>
</tr>
<tr>
<td>++ ++ +</td>
<td>++ ++ +</td>
</tr>
<tr>
<td>Alcoholic precipitate solution, 0.025 cc.</td>
<td>Alcoholic precipitate solution, 0.025 cc.</td>
</tr>
<tr>
<td>++ ++ –</td>
<td>++ ++ –</td>
</tr>
<tr>
<td>Same boiled</td>
<td>Same boiled</td>
</tr>
<tr>
<td>++ ++ +</td>
<td>++ ++ +</td>
</tr>
</tbody>
</table>

+ = degree of fixation of complement.
– = no fixation of complement.

It is seen that the cancer urine (Kittelberger) reacted positively with the blood extract (von Dungern) and negatively with the guinea pig's heart extract (Wassermann). On the other hand, the control urine (Schmidt) reacted positively with the heart extract and negatively with the blood extract, an identical differential result was obtained with the solution of the alcoholic precipitate, and furthermore, it was found that after the solution of the cancer urine alcohol sediment had been boiled, its reactivity with the Wassermann and von Dungern extracts was reversed.

A further series of 98 sera from cases of carcinoma and sarcoma were tested in the General Memorial Hospital according to von Dungern and according to Wassermann; 13 of these were Wassermann positive, and, of the 13, four caused complement fixation also with the blood extract, which was prepared from
washed blood corpuscles from a case of tertiary syphilis. In no other case was complement fixation observed that could be interpreted as a reaction of diagnostic significance.

These experiences with the complement-fixation test for cancer point to the possibility that the positive results that have been reported are due in part to accident and in part to summation effect, or, as Sachs suggests, that a hitherto unknown and uncontrolled factor is required for the successful application of the method.

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