ON COMPLEMENT-FIXATION IN MALIGNANT DISEASE.[1]

By CHARLES E. SIMON and WALTER S. THOMAS.

(From the laboratory of Dr. Charles E. Simon, Baltimore, Md.)

Through the researches of Wassermann and his collaborators it has been established that syphilitic sera may contain substances which in the presence of other substances derived from syphilitic organs are capable of fixing complement, so that upon the subsequent addition of red corpuscles and homologous hemolytic amboceptor hemolysis is impeded. This phenomenon was originally interpreted as meaning that as a consequence of the syphilitic infection specific antibodies are formed which enter into combination with the corresponding antigen—present in syphilitic organs—and that the resultant product is capable of binding complement. Subsequent study has shown that this conception does not satisfactorily account for the facts observed since similar fixation of complement on the part of syphilitic serum may occur in the presence of non-syphilitic tissue extracts. While a different interpretation of the phenomenon must accordingly be sought, the fact remains that substances are formed in the body of syphilitic individuals which will react with certain tissue constituents and bind complement and that the reaction may in a certain measure be regarded as specific. Wassermann states in a recent communication that approximately a thousand cases have been examined, up to the end of the previous year, from which the diagnostic value of the reaction (ergo, its specificity) is uniformly apparent.

Our own work had already been planned at a time when the antigen-antibody interpretation of the syphilitic reaction had not yet been set aside, and when it seemed that the same principle might well be utilized in a search for antibodies in malignant disease. The applicability of the method in the study of pathological conditions, in which the causative agent is unknown, had indeed

[1] Aided by a grant from The Rockefeller Institute for Medical Research. Received for publication May 11, 1908.

been already suggested by Wassermann. At the very outset we were, of course, confronted with the rapidly accumulating facts which go to show that the cancer cell itself may be the parasite proper in malignant disease, and the question naturally suggested itself whether it would be likely under such conditions that any results would be attained.

It seemed that specific antibodies could only be expected, if a specific antigen were operative, and the idea of a specific antigen at first thought appeared likely only if a specific cancer parasite could be assumed to exist. But on further consideration it seemed quite possible that even in the absence of an extraneous factor the cancer cell itself might either give rise to products qualitatively different from those of its normal antecedents, or that, as a result of increased cellular destruction, normal cell products might appear in largely increased amount, and give rise to corresponding antibodies. In either event the cancer cell could be viewed as the antigen and the resultant antibodies would accordingly be auto-antibodies. That auto-antibodies may actually be formed seems to be a well-established fact. It is thus known that auto-hemolysins appear in the serum following the resorption of extravasations of blood. Centanni[2] has demonstrated the appearance of auto-hepato-precipitins in sheep and cattle distomiasis. Bergmann and Salvini[3] have rendered it probable that auto-antibodies are formed in phosphorus poisoning. Schütze and Ascoli[4] succeeded in obtaining auto-precipitins through the injection of homologous albumins, and some observers indeed explain the Wassermann reaction in syphilis upon the basis of auto-antibody formation.[5] The idea that corresponding substances may be formed in malignant disease would accordingly not be far-fetched, and as a matter of fact we have demonstrated that this may actually occur. This

[2] Centanni, E. Contributo alle autocitoreazioni: precipitina e sottrazione del complemento. *Policlinico,* 1906, xiii, 840.—*Idem,* Sulle autocitoprecipitine. *Atti della Societa Italiana di Patologia,* 1906, 404; cited *Jahresbericht über d. Ergebnisse der Immunnitätsforschung,* 1908, ii, 113.

[3] v. Bergmann, S., and Salvini, F. Das hæmolytische Hemmungsphenomen b. Phosphorvergiftung u. anderen pathologischen Prozessen. *Zeit. f. exper. Pathol. u. Therap.,* 1907, iv, 816.

[4] Schütze and Ascoli, cited by Weil, E., and Braun, H. See Note 5.

[5] Weil, E., and Braun, H., Uber Anti-körperbefunde bei Lues, Tabes u. Paralyse. *Berl. klin. Woch.,* 1907, xliv, 1570.

conclusion is warrantable at least upon the basis that the complement fixation method is applicable to decide the question at issue.

TECHNIQUE.

Preparation of the Antigen.—We are aware of the fact that no definite proof has as yet been afforded to show that the reaction is based upon the antigen-antibody principle, and we use the term antigen in this connection merely as a matter of convenience, and to designate in a general way the substance which in the presence of a certain reaction product of the blood serum is capable of fixing complement. Leaving out of consideration, for the present, the question of a specific extraneous cancer parasite, and viewing the cancer cell itself as the essential offending element, which in some manner as yet unknown, gives rise to the formation of corresponding reaction products, it seemed advisable to prepare antigens from different sources and to choose the homologous product in the examination of the individual case, viz., to place the blood serum from a case of cancer of the breast into reaction with breast cancer antigen, the blood serum from a cancer of the stomach with stomach cancer antigen, etc. Upon further consideration, however, we came to the conclusion that it would be wiser at this stage of our research to make use only of tumors in which bacterial infection could be eliminated. Working with extracts from gastro-intestinal cancers it was practically a foregone conclusion that our antigens would also contain large numbers of bacteria, that in the corresponding cases bacteriolysin formation would have taken place, and that any hemolytic inhibition that might be observed could not be attributed exclusively to a cancer reaction. We accordingly made use only of breast cancers, but found that it was necessary to eliminate those in which connective tissue formation stood in the foreground. The common scirrhus is altogether unsuitable as antigen. The best results are obtained with medullary cancers. The tumor material was always obtained at operation, the cancerous portions dissected out, freed from fat as far as possible and ground up with glass in a mortar into a thick paste. This was placed in a suitable receptacle and shaken for about twenty-four hours in a shaking machine with an amount of one-half per cent. carbolic acid

in normal salt solution, sufficient to make an emulsion of moderate consistence. A small amount of thymol was further added as preservative, as the half per cent. carbolic acid is not sufficient to prevent putrefactive changes. The resultant material was kept in the ice-box without removing the saline extract from the tissue. Prolonged exposure to light seems to destroy the activity of the reacting substance, but preserved at low temperature in the dark, we could not detect any loss in strength during a period of at least two months. From the concentrated extract our antigen was freshly prepared on each occasion by contrifugalizing a small amount for at least one hour, at high speed and diluting the supernatant fluid with normal salt solution to a point where no inhibitory effect could be obtained in the presence of the usual amount of *normal* blood serum and fresh guinea-pig complement. This point must be carefully determined, as more concentrated emulsions will by themselves fix complement to an extent that hemolysis may be entirely prevented upon the subsequent addition of the hemolytic amboceptor and corpuscles. The diluted antigen, when ready for use, will of course also fix a certain amount of complement, but the degree of dilution must be so chosen that with normal serum and fresh complement no inhibition of hemolysis will be noticeable after remaining in the incubator for one hour and a half. A determination of the albuminous content of our ultimate dilutions showed about 0.4 per cent. Every antigen before use must, of course, be tested with an appropriate serum, in order to demonstrate that it is capable of inhibiting hemolysis in the presence of such serum (see also below).

The Patient's Serum.—In our work it was not practical for various reasons to obtain the patient's blood from the vein, but we found it perfectly convenient to milk the necessary amount from the ear, after a free puncture with a small lancet or a Hagedorn needle; in some instances larger quantities were obtained at the time of the operation. As the amount of blood at our disposal was smaller than is usually demanded in work of this character we were obliged to make use of smaller quantities also in charging our tubes—0.5 cubic centimeter instead of one cubic centimeter of the diluted serum being our standard amount. This was en-

tirely sufficient, and we would emphasize the value of this method of procuring the blood over venous puncture, especially when many specimens of blood must be procured at one time, and when repeated examinations are to be made. As we were naturally somewhat restricted in the amount of serum, however, in our experiments we worked with single five-fold dilutions of the serum only, tests with diminishing amounts being neglected, and for our purposes unnecessary.

In by far the larger number of our experiments the blood serum was examined within a few hours after the blood was drawn, as Wassermann and his pupils have pointed out that in the syphilitic examinations certain sera may alter their behavior materially on standing, both toward syphilitic and normal tissue extracts. We found, however, that our sera after inactivation for 30 minutes at 52° C. retained their specific activity toward cancer antigen for a number of weeks without impairment whatsoever. We have on hand at present a specimen which was secured seven weeks ago and is as active to-day as when first drawn.

If for any reason our sera could not be examined on the day on which they were obtained, they were separated from the corpuscles as soon as possible; inactivated and then kept, without any preservative, in tightly stoppered tubes in the refrigerator. In several instances carbolic acid was added to the extent of one-half per cent., but we abandoned this, as it appeared that this amount, even after subsequent dilution caused some complement fixation, (sc. destruction).

Our *hemolytic system* consisted of hen corpuscles, anti-hen-rabbit serum, and guinea-pig complement.

The use of hen corpuscles was suggested to us by Dr. R. V. Lamar of the Rockefeller Institute for Medical Research and proved very convenient, as the finer grades of complement fixation can be more readily recognized from the "Schimmer" of the intact corpuscles, which is due no doubt to their form and nucleation. A five per cent. emulsion was employed.

The hemolytic amboceptor was of such strength that .002 cubic centimeter would cause the complete hemolysis of one cubic centimeter of the corpuscle emulsion in the presence of 0.1 cubic centimeter of fresh guinea-pig complement.

The necessity of using fresh guinea-pig complement in our work was really one of our chief difficulties. We frequently attempted its preservation for several days in the frozen state, but found that with our facilities this was almost impossible; even when it had been so kept it happened repeatedly that, although its strength was still sufficient to hemolyze the corpuscles in the presence of hemolytic amboceptor alone, the amount of active substance was so small that the combination antigen-normal serum would absorb nearly the entire amount. We found that it was not safe to use any complement that had been kept longer than twenty-four hours. The neglect of this rule caused the loss of many days' work. We were accordingly forced to sacrifice a new guinea-pig after every two days' work. Puncture of the heart was not practical for our purposes, as we required larger amounts of blood than can be obtained in this manner. Experiments with other sera, as complement, showed that for our system of hen corpuscles and anti-hen-rabbit serum guinea-pig serum alone was applicable. Hog serum, as well as rabbit serum, causes more or less hemolysis of hen corpuscles *per se*. Human serum, chicken serum, and sheep serum are free from this objection, but apparently not rich enough in complement. With human complement particularly, even when combined with normal inactivated serum only, in the absence of any antigen, no hemolysis whatsoever may occur. This in itself is a very interesting phenomenon and would merit more extensive investigation as it seems to throw some light upon the inhibitory reaction which is observed in various pathological conditions, in the absence of any antigen.

The Individual Experiment.—The arrangement of the individual experiment with the necessary controls is apparent from the following schema:

I. *Controls of hemolytic system.*

	Diluted Antigen.	Pts. Serum Dil. 1 : 5.	Normal Serum Dil. 1 : 5.	Complement Dil. 1 : 10.	Amboceptor Dil. 1 : 500.	Corpuscles 5% Emulsion.
1	—	—	—	—	—	0.5
2	—	—	—	—	0.5	0.5
3	—	—	—	0.5	—	0.5
4	—	—	—	0.5	0.5	0.5

			II. *Antigen controls.*			
5	0.5	—	—	—	—	0.5
6	0.5	—	—	0.5	0.5	0.5
			III. *Serum controls.*			
7	—	0.5	—	—	—	0.5
8	—	0.5	—	0.5	0.5	0.5
9	—	—	0.5	—	—	0.5
10	—	—	0.5	0.5	0.5	0.3
			IV. *Serum-antigen controls.*			
11	0.5	0.5	—	—	0.5	0.5
12	0.5	0.5	—	0.5	—	0.5
13	0.5	—	0.5	0.5	0.5	0.5
14	0.5	—	0.5	—	0.5	0.5
15	0.5	—	0.5	0.5	—	0.5
			V. *Experiment proper.*			
16	0.5	0.5	—	0.5	0.5	0.5

The tubes which contain antigen-complement, serum-complement, or antigen-serum and antigen-serum-complement are incubated for one hour at 37° C. before the corpuscles and the hemolytic amboceptor are added; when two components only are combined one volume of normal salt solution (0.85 per cent.) is added and when one only is employed two volumes of saline solution are used. After the addition of the hemolytic system (viz., amboceptor and corpuscles) the tubes are returned to the incubator, the corpuscles shaken up at intervals of about fifteen minutes, and careful note taken of the progress of the hemolysis.

The most important controls, of course, in judging the progress of the hemolysis, are Numbers 8 and 13; these, besides Numbers 4, 6 and 10 should invariably be made. The others can be neglected in future cancer work, as our studies have shown that they are unnecessary, if the technique here advocated is followed; if any deviations, however, are contemplated they should all be carefully considered.

Wassermann originally advocated that the tubes, after the addition of the hemolytic system, be left in the incubator for two hours, that they should then be removed to the ice-chest and examined the following day. This seems to us entirely too arbitrary, considering the fact that we are working with several factors of

which two at least are inconstant quantities, viz., the patient's serum and the complement content of the guinea-pig serum, which certainly differs more or less in different animals. We would merely recall the loss of complement which occurs during the process of fasting. Neglect of this factor on one occasion caused us the loss of a whole day's work. We trust that ere long we may be in a position to control quantitatively the exact amount of complement that is added, but until this can be done we are scarcely justified in setting an arbitrary time limit, during which the specimens are to be kept in the incubator. There are sera, it is true, which in the presence of suitable antigen bind complement in so large a quantity that one could safely leave the tubes in the incubator for many hours without the occurrence of any hemolysis whatever, but it is more common to meet with smaller amounts of complementophilic substance, in which hemolysis is merely delayed, as compared with the controls 8 and 13. In order to demonstrate this delayed hemolysis, which, of course, must be interpreted as a certain grade of fixation, it is absolutely necessary to observe the specimens from time to time in the incubator and to regard the experiment as ended, for a given specimen, as soon as the controls show that hemolysis in these is complete. In many cases this point is reached after an hour or an hour and a quarter.

The thought naturally suggested itself that the existence of a moderate increase of complement fixation beyond normal could be demonstrated more readily, if smaller amounts of complement were used. This, however, we found impractical as the degree of complement fixation on the part of the normal sera is at times so great that the upper limit of the normal and the lower limit of the abnormal were thus brought fairly close together. To be sure, the lower limit of the abnormal seems to be even then so far above the normal, that it is possible to demonstrate the increase in most cases by the size of the "Kuppe" in the centrifugated specimen, or by the hemoglobin content of the supernatant fluid, as determined with Fleischl's haemometer, for instance. But our technical difficulties were sufficiently numerous without additional complications, so that we were content at this stage of our research to confine our attention to gross reactions and to abandon the

attempt to obtain a maximal "Ausbeute" of positive findings. For this reason especially did we continue the use of the larger amounts of complement, and attempt to overcome the resultant difficulties by disregard of an arbitrary time limit. That Wassermann and his collaborators have since come to a similar conclusion in their syphilitic work is clear from the paper of Meier,[6] and suggests itself as natural to anyone who has worked along these lines. The tubes were accordingly examined from time to time and the experiment interrupted as soon as the controls 8 and 13 showed complete hemolysis. In the majority of our cases no difficulty was experienced in deciding whether fixation had occurred or not; in some instances there was no trace of hemolysis, but as a rule partial fixation only was obtained in the positive cases; if any doubt was felt whether there was any fixation at all the specimens were centrifugalized, when the question was readily settled.

RESULTS.

While many more cases were examined than are included in the accompanying tables we have taken special pains to eliminate every observation concerning which the least doubt was felt. The list hence includes only those cases in which a well pronounced reaction was either present or absent, viz., those in which it was perfectly clear that complement fixation had or had not occurred.

A study of the tables shows at once that in the malignant cases fixation occurs quite frequently, while in the non-malignant cases it is rare. In our list which includes the most diverse pathological conditions it was indeed observed but twice and in these the fixation finds a probable explanation in the history of the patient. No. 49 was admitted to the hospital with advanced tertiary syphilis (necrosis of the turbinates and septum) and we are strongly inclined to attribute the positive reaction to this condition, since Weil and Braun have shown that the syphilitic reaction may also be obtained, if tumor extracts are used as antigen instead of extracts from syphilitic livers. In the second case, No. 42, a young colored

[6] Meier, G. Die Technik, etc., der Wassermannschen Reaction auf Syphilis. *Berl. klin. Woch.*, 1907, xliv, 1636.

TABLE I.

NON-MALIGNANT CASES.

1. G. G.	Normal	Complete hemolysis.	
2. Mr. H.	Gallstones	"	"
3. Mr. Z.	Gallstones	"	"
4. Mrs. G.	Calcified fibroid	"	"
5. Mr. J.	Empyema	"	"
6. Mr. N.	Gangrene of the foot (diabetic)	"	"
7. Mr. S.	Renal calculus	"	"
8. Mr. McL.	Chronic cystitis, nephritis	"	"
9. Mrs. St.	Benign tumor of parotid	"	"
10. Mrs. B.	Hysteria	"	"
11. Mr. K.	Tubercular sinus of the hip	"	"
12. Mr. X.	Amputation at the wrist	"	"
13. Mr. X.	Gonorrhœal arthritis	"	"
14. Mr. W.	Tuberculosis of the hip joint	"	"
15. Mr. Y.	Frost bitten feet	"	"
16. Mr. B.	Typhoid fever, convalescent	"	"
17. Mrs. P.	Septicæmia following abortion	"	"
18. Mr. W.	Erysipelas	"	"
19. Mrs. St.	Renal tuberculosis	"	"
20. Mrs. W.	Appendicitis and peritonitis	"	"
21. Mrs. E.	Suppurating appendicitis	"	"
22. Mr. X.	Uræmia	"	"
23. Mr. X.	Perihepatic abscess	"	"
24. Mr. G.	Suppurating appendicitis and perinephritic abscess	"	"
25. Mrs. N.	Inflammatory mass in pelvis	"	"
26. Mr. McG.	Hepatic cirrhosis and chronic pancreatitis	"	"
27. Mrs. B.	Suppurating tubercular case	"	"
28. Mr. H.	Cardiac lesion	"	"
29. Mr. G.	Hysteria	"	"
30. Mr. P.	Tubercular sinus	"	"
31. Mr. A.	Typhoid fever	"	"
32. Mrs. L.	Cyst of the broad ligament	"	"
33. Mrs. G.	Ovarian cyst	"	"
34. Mr. A.	Gonorrhœal rheumatism	"	"
35. D.	Compound fracture of radius	"	"
36. E.	Pneumonia, a few days after crisis	"	"
37. M.	Fracture of malar bone	"	"
38. T.	Pleurisy with effusion	"	"
39. W. T.	Normal	"	"
40. Mrs. S.	Normal	"	"
41. C. E. S.	Normal	"	"
42. L. C.	Tubo-ovarian abscess (fixation before operation); after operation	"	"
43. Mr. R.	Myeloid myelocytic leukemia	"	"

44. Mrs. F.	Normal	Complete hemolysis.
45. M. W.	Procidentia of the uterus	" "
46. Miss B.	Pneumonia (convalescent)	" "
47. Mrs. K.	Pseudoleukemia	" "
48. Mr. St.	Appendicular abscess	" "
49. F.	Alcoholism	Fixation.
50.	Pooled serum of four normal persons	Complete hemolysis.

TABLE II.

Malignant Cases.

1. Mrs. H.	Carcinoma of the liver	Fixation.
2. Miss T.	Carcinoma of the rectum, p. o.	"
3. Mrs. R.	Carcinoma of the uterus, p. o.	"
4. Mrs. C.	Carcinoma of the breast, p. o.	"
5. Mrs. O.	Carcinoma of the uterus, p. o.	Hemolysis.
6. Mrs. R.	Carcinoma of the uterus, p. o.	"
7. Mrs. F.	Carcinoma of the uterus, p. o.	"
8. Mrs. B.	Carcinoma of the breast, p. o.	Fixation.
9. Mr. X.	Carcinoma of the prostate, p. o.	"
10. Mrs. S.	Carcinoma of the breast, p. o.	"
11. Mr. K.	Malignant growth of the lower jaw	"
12. Mrs. P.	Recurrent tumor of the parotid, a. o.	"
13. Mrs. B.	Carcinoma of the rectum, p. o.	"
14. Mrs. H.	Small scirrhus of the breast, p. o.	Hemolysis.
15. Mr. G.	Recurrent carcinoma of the thyroid, a. o.	"
16. Mrs. L.	Carcinoma of the breast, p. o.	Fixation.
17. Mr. C.	Melanotic sarcoma	Hemolysis.
18. Mr. D.	Carcinoma of the stomach	"
19. Mrs. D.	Carcinoma of the breast	Fixation.
20. Mr. W.	Carcinoma of the pancreas	Hemolysis.
21. Mr. W.	Carcinoma(?) of the submaxilary gland	"
22. Mr. H.	Carcinoma of the stomach	"
23. Mr. H.	Carcinoma of the stomach	Fixation.
24. Mr. F.	Carcinoma of the common duct and gangrenous cholecystitis	"
25. Mrs. F.	Carcinoma of the uterus, p. o.	"
26. Mrs. C.	Carcinomatous degeneration of a uterine fibroid	" "
27. J. R.	Carcinoma of the stomach (?) (absence of HCl, presence of lactic acid)	"
28. Mrs. B.	Carcinoma of the rectum, p. o.	"
29. A. B.	Carcinoma of the ovary, p. o.	"
30. Mr. K.	Carcinoma of the prostate, p. o.	"
31. Miss H.	Carcinoma of the breast (recurrent), p. o.	Hemolysis.
32. L.	Carcinoma of the stomach	"
33. Mr. H.	Carcinoma of the stomach	"
34. Mrs. B.	Carcinoma of the breast, p. o.	Fixation.

35. Mr. K.	Carcinoma of the œsophagus.....	Fixation.
36. Mrs. W.	Carcinoma of the uterus, p. o.	"
37. L. P.	Carcinoma of the stomach (?).....	"

p. o. indicates post operative; a. o. before operation.

woman, a direct syphilitic history could not be obtained, but the fact that she was operated for pus tubes suggested that the possibility of a preceding syphilitic infection can at least not be denied. Unfortunately we did not have appropriate syphilitic antigen at hand to test this question at the time.

It might of course be argued that since cancer antigen may cause complement fixation with syphilitic serum that some of our positive findings in the malignant column also may have been due to syphilis and not referable to the existence of malignant disease.

If then we eliminate the known case of syphilis from the non-malignant column and allow the doubtful one to remain we find that with cancer antigen no fixation occurred in 98 per cent. of all cases, while among the cancer cases 65 per cent. gave a positive reaction.

Considering the fact that we have thus far worked only with breast cancer antigen, and that the method is as yet of necessity imperfect, it must seem not unreasonable to suppose that with improved technique a still larger yield of positive findings may be obtainable, and that the reaction may become an important one in diagnosis. While we are aware of the fact that the number of cases which we have had occasion to examine is still far too small to permit any far reaching conclusions, we feel strongly tempted to state even now that barring the occurrence of syphilis a positive reaction with cancer antigen may be regarded as strong evidence of the existence of malignant disease. Our blood specimens came to us frequently, merely numbered, or labelled with the name of the patient and without any clue regarding the nature of the disease; still we were frequently able to make the diagnosis without having any previous knowledge of the patient's condition whatsoever. As an example we would cite the following instance: We have pointed out before that while in the majority of cases the complement fixation is only partial it may at times be complete. This complete reaction is so striking that it naturally attracts imme-

diate attention. We were accordingly much interested to find this in a specimen of blood which came to us with the patient's name, but without further comment. Upon inquiry whether the case were a malignant one we were told that the patient had only been admitted that morning, that there was a "lump" connected with the lower jaw, but that no further data had been obtained. The history then showed the following:

The patient was a man, æt. 70, whose general health had been excellent; there was in particular no history of syphilis; his wife had died of typhoid fever and there were six children living and in good health. About a week before Christmas, 1907 (ten weeks before admission) he first noticed a swelling in the right lower jaw which gave rise to much pain, and became rapidly larger. Upon admission to the service of Dr. Wm. Fisher, its size was about that of a small egg, over which the skin was partly adherent. It was irregular in form and extended along the floor of the mouth in the region of the submaxillary gland. Its outline was indefinite, but seemed partly connected with the bone; it was hard to the touch, decidedly tender on deep palpation, and not connected with any teeth, which had all been removed on that side many years ago. The tumor was removed by Dr. Fisher together with the ramus of the jaw and the submaxillary gland, and submitted to Dr. Bloodgood for examination, who reported that the diagnosis lay between alveolar sarcoma, endothelioma and carcinoma.

This case is particularly interesting, as the possibility exists that it is a connective tissue tumor and that the reaction was obtained with breast cancer antigen. Our list includes two other tumors of this order, Nos. 26 and 29, viz., a sarcomatous fibroid and an ovarian sarcoma.

We realize that it would be unwarrantable to draw any far-going inferences from these observations, but the finding is especially interesting since it has been shown that mice which have been rendered immune to carcinoma are also immune to sarcoma, in other words, a much closer relation must exist between the connective tissue tumors and epithelial growths than was formerly supposed.

It will be noted from a survey of our cases that in many the examination was made after operation, the interval varying between a day and several weeks. The question accordingly arises whether some of our negative results may not be owing to the fact that the blood was taken relatively late after the extirpation of the growth. While this possibility cannot be eliminated without

further investigation we surmise from our observations that the time element, within certain limitations, is not responsible for the negative findings, for we have been able to ascertain that the "inhibitory" substance does not necessarily disappear from the blood even after extensive excision, and we have even gained the impression that the amount does not necessarily diminish. In some instances, it is true, the reaction becomes materially less marked after a number of weeks and may even disappear, but in others, after a temporary decrease in its intensity, it was later found as marked as in the beginning. In Case 11, for example, about three weeks after operation, the reaction was just as intense as the day preceding the removal of the growth. How long it may remain present after operation future studies will have to show.[7]

We are thus not inclined to attribute our negative findings to the removal of the growth, but rather believe that the reaction was absent also before operation, and on considering the location of the growths in the negative cases the thought suggests itself that in these auto-inoculation with the primary degenerative products of the tumor may not occur so readily as in the others. For it will be noted that the majority of these negative cases are growths which are connected with the channels of the body which lead directly to the exterior, viz., the gastro-intestinal canal and the vagina. This question, however, cannot be decided until a much larger amount of material has been studied and classified. We mention the matter merely as a possibility. Apparently opposed to this explanation is the fact that continued vaccination of patients with shake extracts of cancer tissue does not necessarily cause the reaction to appear, when it has been absent before. Our observations, however, here also, are still too small in number to allow us to speak definitely, and the time during which the injections have been continued too short. We hope to report on this point in detail on a future occasion.

It would of course be tempting to theorize on the basis of our observations, but we purposely refrain from doing so, and are content in merely stating the facts, which have been obtained. The en-

[7] A recent examination of this case, about eight weeks after operation, showed only a very faint reaction.

tire question of complement fixation as the result of an "antigen-antibody" reaction is hardly yet open to intelligent speculation, and we would emphasize once more that we have used the term antigen and antibody in the present paper merely as a matter of convenience and do not wish to imply in the least that we regard the reacting substance in the blood serum as the expression of a defensive reaction on the part of the body, and hence as a measure of cancer immunity. We incline to the view, however, that the serum reaction product is "tuned" more or less specifically to the cancer antigen and would emphasize that negative reactions only, with cancer antigen, have been obtained in the numerous cases in which "normal" body cells were undergoing degeneration even in large numbers, as exemplified more specifically by the negative findings in the myeloid myelocytic leukemia, and in the various types of abscess formation, as noted in our first table.

While the complement fixation to which reference has been had in the foregoing pages depends upon the interaction between two factors, of which one is present in the blood serum of the patient and the other in the cancer extract, we have met with a second type, in which the inactivated serum binds complement *per se*. In our series of nearly one hundred cases this occurred but four times, viz., in two cases of hepatic cirrhosis, in one of Banti's disease, and in a patient who had passed through three operations for cancer of the breast and had been treated for six months with injections of a cancer vaccine. This phenomenon was first observed by Neisser and Doering[8] in 1901 in a case of impending uremia and has since been studied by Neisser and Friedemann,[9] Laqueur,[10] Hedinger,[11] Wolze,[12] Senator,[13] Lüdke,[14] and especially by v. Berg-

[8] Neisser and Doering, Zur Kentniss d. hæmolytischen Eigenschaften d. menschlichen Serums. *Berl. klin. Woch.*, 1901, xxxviii, 593.

[9] Neisser and Friedemann, Uber Amboceptoidwirkung in einem menschlichen Serum, *ibid.*, 1902, xxxix, 677.

[10] Laqueur, Zur Kentniss uræmischer Zustände. *Deutsch. med. Woch.*, 1901, xxvii, 744.

[11] Hedinger, Klin. Beiträge z. Frage d. Hæmolyse. *Deutsch. Arch. f. klin. Med.*, 1902, lxxiv, 24.

[12] Wolze, Zur Hemmung d. Hæmolyse b. uræmischen Zuständen. *Cent. f. inn. Med.*, 1903, xxiv, 649.

mann and Keuthe.[15] It has been met with in isolated cases of pneumonia, sepsis, carcinoma, leukemia, uremia, etc., and has been experimentally produced by v. Bergmann and Salvini in phosphorus poisoning, and by Eva Hoffman[16] in uremia and nephritis.

Since Neisser and Friedmann found a distinct decrease in the amboceptor content of the heated serum they attempted an explanation of the phenomenon by assuming the formation of amboceptoids and their union with the haptophoric groups of the red corpuscles. V. Bergmann and Keuthe, however, showed that hemolytic inhibition occurred also, if previous to heating, the amboceptors of the serum are removed by uniting them with red corpuscles and centrifugalizing, after absorption of complement by means of yeast. They accordingly conclude that hemolytic inhibition is referable to preëxistent anticomplement, which in active serum is bound by a certain amount of complement, and that as the result of heating to 56° C. the binding complement is destroyed and the anti-complement liberated. From their experimental work in phosphorus poisoning v. Bergmann and Salvini are further led to assume that in the degenerating liver substances are liberated that lead to the production of antibodies. If then both antigen and antibody are simultaneously present in the circulation, in considerable amount, the result is an impoverishment of complement, which can be directly demonstrated. If the combination antigen-antibody is present in smaller quantity the inhibitory effect may still be demonstrable in the *inactivated* serum and may be increased by the further addition of antigen. V. Bergmann and Salvini finally suggest that it might be possible to demonstrate the inhibition by the addition of a suitable amount of antigen even in cases in which, without this, hemolysis must proceed unimpeded. This explanation it

[13] Senator, Uber d. hæmolytische Eigenschaft d. Blutserums b. Uræmie. *Berl. klin. Woch.*, 1904, xli, 181.

[14] Lüdke, Beit. z. Studium d. Complemente, *Münch. med. Woch.*, 1905, liii, 2065 and 2126.

[15] v. Bergmann and Keuthe, Die Hemmung d. Hæmolyse durch inactivirte menschliche Sera, *Zeit. f. exper. Pathol. u. Therap.*, 1906, iii, 225.

[16] Hoffmann, Experimentelle Untersuchungen über d. hemmende Wirkung inactivirter Sera., *ibid.*, 704.

seems to us, may be applicable also in certain cases of cancer, as well as in the syphilitic cases and certainly deserves careful consideration. But it does not satisfactorily explain the continued inhibition after operation, unless we assume that its persistence is an indication that the cancerous tissue has not all been removed. We hope to report our own investigations in this direction in a future paper.

What relation, if any, exists between these findings under pathological conditions and the thermostabile anticomplementary constituants of normal blood serum of Noguchi[17] and Manwaring[18] future investigations will have to show. A demonstration of their identity would of course constitute a very serious obstacle to the acceptance of v. Bergmann's view.

[17] Noguchi, H. The Thermostabile Anticomplementary Constituents of the Blood. *Journ. of Exper. Med.*, 1906, viii, 726.

[18] Manwaring, W. H. The Third Serum Component. *Journ. of Infect. Diseases,* 1906, iii, 647.