USE OF SCHULTZ-DALE TEST FOR DETECTION OF SPECIFIC ANTIGEN IN SERA OF PATIENTS WITH CARCINOMA

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Early diagnosis is still the keystone for effective treatment of cancer in the majority of cases. It is therefore not surprising to find a very long list of attempts at diagnostic procedures for cancer. Homburger (1950) lists a number of these tests, including those with and without some established value. The great majority of these tests, however, are not specific and have failed to fulfil the requirements put up by Dunn and Greenhouse (1951) of no more than 10% false negatives and not more than 5% false positives.

Although the morphological methods used, including the comparatively new method of Papanicolaou, still provide the basis of diagnosis, they have, as Homburger points out, many drawbacks. Apart from the necessity for highly skilled personnel, the Papanicolaou technique is useful only in those lesions where the cells can be scraped from the surface, or that are freely accessible to liquids which can wash away desquamated cells and bring them within the reach of the examiner.

The need is therefore great for a specific test which could utilize a more general common characteristic of neoplasms, and which could be applied to the blood or body secretions, for the detection of all types or any given type of cancer.

The utilization of immunological procedures to detect specific changes in the blood of patients with cancer would seem to be promising in its approach to the problem. Numerous tests based on immunological reactions have been described, but have not been proved of practical value. A number of these tests have been listed (Homburger, 1950; and U.S. Public Health Service, 1951, 1953). The immunological blood tests described are all concerned with detection of antibodies.

It has been found by Schultz (1910) and Dale (1913) that when the sensitized guinea-pig intestine or uterus is removed from the body and suspended in oxygenated Ringer's solution, maximal contraction will occur on exposure to even small amounts of antigen. Using the Schultz-Dale reaction, Makari (1953) was able to detect rickettsial antigen in egg yolk and cotton-rat liver. Similarly, Makari and Mirick (1955) were able to detect a soluble antigen in sera of patients with infective hepatitis and homologous serum jaundice. Using the same method, Makari (1955a, 1955b) described the presence of a soluble carcinoma antigen.

The present preliminary report concerns the use of the Schultz-Dale technique for the detection of a soluble antigen in the sera of patients with carcinoma, and the possible application of this finding for diagnosis of carcinoma in man. A more detailed report will be published elsewhere.

Materials and Methods

Virgin female guinea-pigs weighing about 300 to 400 g. were immunized to antigens from carcinomatous tissues by the help of Freund's adjuvants. After four weeks they were killed by a blow on the head, and their uterine horns were removed and tested in a modified Schultz-Dale apparatus shown in Fig. 1.

The Tyrode solution used was the one suggested by Coulson *et al.* (1943), with the calcium chloride content diminished to 0.05 g. per litre. A mixture of 95% oxygen and 5% carbon dioxide was bubbled through. Air has proved satisfactory in more recent work. Recordings were done with ink on B.M.R. calibrated paper mounted on rotating kymographs. A run lasted for five minutes, and covered a total of 10 cm. on the drum.

Before the addition of the serum to be tested, the horn was allowed to remain in the Schultz-Dale bath long enough

FIG. 1.—Schultz-Dale apparatus as modified for use in this work. A=Water-bath at 37.5° C. B=Tyrode reservoir. C=Schultz-Dale bath. (1) Uterine horn. (2) Glass rod for attachment of lower end of horn. (3) Needle for bubbling 95% oxygen and 5% carbon dioxide. D=Test-tube holder. E=Sterile test-tubes with serum or plasma to be tested. F=Kymographs. (a) B.M.R. paper. (b) B.M.R. ink-tip.

to ensure complete relaxation. A horn from an unsensitized normal guinea-pig was used as a control. Several antigens were used to immunize these guinea-pigs: *Antigen A* was prepared by homogenization in a Waring blender of carcinomatous tissue under sterile precautions, which was then made a 20% emulsion in normal saline. *Antigen B* was prepared from the HeLa strain of cancer cells grown in tissue culture in Dr. Gey's laboratory (Gey and Gey, 1949; Gey *et al.*, 1952). *Antigen C* was the supernatant obtained after centrifugation of antigen A at 3,200 r.p.m., equivalent to an R.C.F. of 1.5 kg. per g. *Antigen C* was further fractionated by centrifugation to Sediment II, obtained between 3,200 and 4,000 r.p.m., equivalent to an R.C.F. of 2.3 kg. per g., and Sediment III, between 4,000 and 10,000 r.p.m., equivalent to an R.C.F. of 10.3 kg. per g.

The testing procedure was started with the addition of 0.5 ml. of normal Group O plasma to the Schultz-Dale bath, which did not result in contraction of the normal horn, but caused a specific contraction of the sensitized horn, due to a common component between the cancer antigens and normal Group O plasma. Further additions of normal Group O blood in 0.5-ml. amounts were made until the horns did not record any contraction on contact with the Group O plasma for five minutes (one run), indicating complete desensitization of the sensitized horns to antigens in normal blood.

After each addition of serum, the Tyrode solution in the Schultz-Dale bath was changed a sufficient number of times (three or four) to allow the return of the kymograph reading to the baseline.

After desensitization to Group O was accomplished, any serum or plasma which gave a positive reaction in the sensitized horn, but not in the normal horn, was considered a positive reaction. Any serum which caused a contraction of

the normal horn could not be evaluated. Only contractions which were at least one-fifth as strong as that produced by 10 gamma of histamine base were considered as significant. The record of a typical experiment is shown in Fig. 2.

Serum obtained from guinea-pigs or rabbits four to six weeks after primary immunization, or one to two weeks after secondary immunization, was used for

FIG. 2.—Record of a typical experiment on guinea-pig 56, Group 20, actively sensitized to FrO antigen C on November 12, 1953, and sacrificed a month later.

passive sensitization. Normal uterine horns were passively sensitized by contact with such serum for one hour at 4° C. Horns passively sensitized by this treatment may be used in the Schultz-Dale bath in the same way as the actively sensitized ones.

The sera tested were collected from patients at the Johns Hopkins and Baltimore City Hospitals. The diagnoses were as follows: definite carcinoma, questionable carcinoma (including hyperplasias), other malignant or benign tumours, and non-neoplastic diseases. The cases of carcinoma or

other tumours were divided into untreated cases and those which had received radiotherapy and/or surgery. Presumably normal individuals from the Cancer Detection Clinic at the Johns Hopkins Hospital were also studied.

Individuals of both sexes were used throughout the study, and the age range was 35 to 65 years. A total of more than 1,000 sera were studied from 654 persons by this test. An additional 47 patients were studied for the presence of a pressor substance only, and an additional six for platelet effect. Reproducibility of the test was studied by means of plasma pooled from three cases of carcinoma or plasma pooled from three normal Group O individuals.

The conditions under which the serum was collected and stored had considerable influence on the outcome of the test, and this phase of the investigation will be described in detail elsewhere. Unless otherwise specified, serum was stored for 24 hours at 4° C. before being used. If not used immediately, further storage was at -40° C.

Sensitivity and Specificity of Test as Related to Immunizing Antigen

The results of the Schultz-Dale test on sera from 517 individuals are shown in Table I and Fig. 3.

With antigens A and B, active sensitization resulted in a positive test in 59 out of 70 (84%) cases of untreated carcinoma, and passive sensitization yielded a positive result in 18 out of 21 (86%) such cases. The incidence of positive reactions in questionable cancer cases was 11 out of 18 with active sensitization, and five out of five with passive sensitization. Two out of 10 cases of tumour other than carcinoma gave positive reactions with active sensitization and two out of four with passive sensitization.

The incidence of positive reactions among individuals from the Cancer Detection Clinic was 5% (6 out of 111) with active sensitization, and 4% (1 out of 26) with passive sensitization. In cases with other diseases 19 out of 23 (82%) were positive with active sensitization, and 8 out of 25 (32%) were positive with passive sensitization.

When antigen C was used—that is, the antigen with small-sized particles—the sensitivity and specificity of the test were

TABLE I.—Distribution of Positive Reactors to Schultz-Dale Test among Untreated Carcinoma Cases; Cases with Questionable Carcinoma, other Tumours, and other Diseases; and Presumed Normals

Source of Antigen	Type of Antigen Used	Type of Sensitization	Positive Carcinoma		Questionable Carcinoma		Other Tumours		Other Diseases		Presumed* Normals	
			Total Cases	Positive Cases	Total Cases	Positive Cases	Total Cases	Positive Cases	Total Cases	Positive Cases	Total Cases	Positive Cases
Carcinoma of ovary H. S. or HeLa strain tissue culture	Antigens A and B (large- and small-sized particles)	Active	70	59 (84%)	18	11 (61%)	10	2 (20%)	23	19 (82%)	111	6 (5%)
		Passive	21	18 (86%)	5	5 (100%)	4	2 (50%)	25	8 (32%)	26	1 (4%)
Carcinoma of sigmoid C. H. or Carcinoma of bronchus M. J.	Antigen C (small-sized particles)	Active	64	62 (96.8%)	8	3 (37.5%)	9	1 (11%)	123	6 (4.8%)	—	—

* Individuals tested from the Cancer Detection Clinic at the Johns Hopkins Hospital.

FIG. 3.—Percentage distribution of positive reactors to the Schultz-Dale test in 313 cases where antigens A and B were used for sensitization (left), and in 204 cases where antigen C with small-sized particles was used (right).

greatly increased. With the active sensitization technique the incidence of positive reactors in the definite carcinoma cases was 62 out of 64 (96.8%); in the questionable cancer cases, 3 out of 8; in other tumours, 1 out of 9; and in other diseases, 6 out of 123 (4.8%).

Untreated Carcinoma.—Results obtained with active sensitization in the 134 patients with untreated carcinoma are listed in Table II with regard to the site of the carcinomatous process. It is apparent that nearly all carcinomas

TABLE II.—*Distribution of Sites of Origin in 134 Untreated Carcinoma Cases Tested by Active Sensitization with Schultz-Dale Test*

Source of Carcinoma	Antigens A and B (H. S. and HeLa Strain)		Antigen C (C. H. or M. J.)	
	Total	Correct Diagnosis	Total	Correct Diagnosis
Abdominal cavity ..	—	—	4	4
Bladder ..	6	6	1	1
Bone ..	1	1	—	—
Brain ..	—	—	2	2
Breast ..	1	1	4	4
Bronchi ..	—	—	2	2
Cervix ..	7	7	1	1
Colon ..	1	1	1	1
Generalized (metastasis) ..	6	3	9	9
Larynx ..	—	—	4	4
Lung ..	1	0	4	4
Mediastinum ..	—	—	1	1
Neck ..	—	—	1	1
Oesophagus ..	2	2	2	2
Ovary ..	1	1	—	—
Pancreas ..	1	1	2	2
Penis ..	1	1	—	—
Pharynx ..	—	—	2	0
Prostate ..	15	10	1	1
Recurrent ..	1	1	1	1
Rectum ..	—	—	1	1
Site not recorded ..	15	14	16	16
Skin ..	1	1	—	—
Stomach ..	3	3	2	2
Testes ..	—	—	1	1
Tongue ..	4	4	—	—
Thyroid ..	2	1	—	—
Uterus ..	1	1	2	2
	70	59 (84%)	64	62 (96.8%)

tested from the 28 sites listed have antigens in common with the carcinomas used in preparing the antigens employed for sensitization. From these findings one might reasonably postulate the presence of common antigens among all carcinomas. Such a common antigenicity would provide a rational basis for immunological detection of carcinomas, using a single antigen. It will be noted that the proportion of false negatives was largest with antigen B prepared from the HeLa strain of carcinoma. Of the false negatives obtained with antigen B, 8 out of 11 were from patients with carcinomas of the prostate or metastatic carcinoma. These findings suggest a possible loss of antigenicity through adaptation of the HeLa strain to its abnormal environment in tissue culture. This loss may selectively involve one or more antigens, and may be related to the capacity of tumour cells to metastasize.* The two cases giving false-negative results with antigen C were both carcinoma of the pharynx, a finding for which no explanation is apparent.

Carcinoma Treated with Surgery or Radiotherapy.—When guinea-pigs actively sensitized to antigens A and B were used to test sera from surgically treated carcinoma patients during their first post-operative week, four out of six gave positive results, and one out of three gave a positive reaction with sera obtained after the first week of operation. With antigen C one out of four gave a positive reaction with sera taken in the first post-operative week. With sera from patients who had received radiotherapy within the previous week, four out of four cases were positive. In another case the test was negative seven weeks after radiotherapy. The number of cases studied following therapy is too small for any evaluation.

*After this article was prepared, Dr. H. N. Green's article (*British Medical Journal*, 1954, 2, 1374) on an immunological concept of cancer was noted, which supports this idea.

Other Tumours.—In Table III are shown results obtained with 19 cases having tumours other than carcinoma. Two cases of sarcoma, mentioned below in the questionable group and giving negative results, are not included. Of the two patients showing positive results with antigens A and B, one had a melanoma and the other a seminoma.† The negative

TABLE III.—*Incidence of Positive Reactors to Carcinoma Antigens in the Schultz-Dale Test in Other Tumours than Carcinoma*

Type of Tumour	Antigens A and B		Antigen C	
	Total	Positive	Total	Positive
Carotid tumour ..	—	—	1	0
Hodgkin's disease ..	1	0	3	0
" sarcoma ..	1	0	—	—
Leukaemia ..	1	0	2	0
Lymphoma ..	1	0	—	—
Lymphosarcoma ..	1	0	—	—
Melanoma ..	2	1	1	1
Myoma ..	—	—	1	0
Reticulum-cell sarcoma ..	1	0	—	—
Sarcoma ..	1	0	1	0
Seminoma ..	1	1	—	—
	10	2	9	1

cases included four sarcomas, a lymphoma, a case of leukaemia, and a case of Hodgkin's disease. With antigen C only one of nine cases was positive, and this was also a melanoma. The negatives included two leukaemias, three cases of Hodgkin's disease, one sarcoma, one carotid body tumour, and one uterine myoma. It is of interest to note that of the three melanomas studied two were positive, indicating a common antigenic component perhaps with carcinoma, and so was the case of seminoma studied. The cases of sarcoma, Hodgkin's disease, and leukaemia studied did not show any common antigenicity with the carcinoma antigens used, as evidenced by the negative reactions obtained.

Other Diseases.—With guinea-pigs actively sensitized to antigens A and B, 19 out of 23 cases of non-neoplastic disease gave positive tests. Since sera from the positive cases were not tested with unsensitized horns, it is not apparent whether positives were due to a pressor substance and/or a common antigen.

Of the 123 cases studied with guinea-pigs actively sensitized to antigen C, 6 gave positive results when tested with unsensitized horns, indicating the presence of a pressor substance, and thus could not be evaluated.

Cancer Detection Clinic.—A total of 111 apparently healthy adults of both sexes were studied by the active sensitization method with antigens A and B. Of these, six were positive reactors. These six were also shown to have pressor substances when tested with unsensitized horns. When these cases were being studied the thermolability of this pressor substance and the use of platelet intact plasma had not been established. It was therefore not known whether or not these positive results were false positives in the usual sense.

Follow-up of Cases with Clinical Diagnosis of Questionable Carcinoma

The cases in this group were followed for a period of one year. Of the 26 cases, the 9 in which a definite pathological diagnosis was obtained at necropsy or by biopsy are reported in Table IV.

On subsequent pathological examination a diagnosis of carcinoma was not obtained in any of the four cases showing a negative reaction, the pathological diagnoses being fat necrosis, lupus erythematosus, haemangiosarcoma, and spindle-cell lipo-fibrosarcoma.

Of the five cases with a positive reaction, four proved to be definite carcinoma. The fifth case, J. C., had a recurrent papilloma of the bladder, which was found at operation a day later to be penetrating the bladder wall, suggesting beyond doubt its carcinomatous nature.

†While the question of melanoma is still a debatable issue, the great majority of workers feel that since the seminoma is a tumour of germinal epithelium it is best grouped under carcinoma.

TABLE IV.—*Follow-up of Cases with Questionable Clinical Diagnosis of Carcinoma*

Initials	Diagnosis at Time of Testing	Result of Test	Final Diagnosis by Biopsy or Necropsy	Interval Between Test and Definite Pathological Diagnosis in Days
M. C. ..	? Carcinoma of bladder	+	Carcinoma (bladder)	28
A. G. ..	? Carcinoma of pancreas	+	Carcinoma (head of pancreas)	10
J. S. ..	? Carcinoma of lung	+	Undifferentiated carcinoma of lung	4
G. M. ..	? Carcinoma of gums (recurrent)	+	Extensive carcinoma (recurrent)	151
J. C. ..	Papilloma ? carcinoma of bladder	+	Papillary squamous-cell tumour with invasion of bladder wall	1
A. B. ..	? Carcinoma of breast	—	Fat necrosis (no carcinoma)	14
A. R. ..	? Carcinoma of lymph node	—	Lupus erythematosus (no carcinoma)	20
J. Y. ..	? Carcinoma of stomach	—	Haemangiosarcoma (tail of pancreas)	56
E. C. ..	? Carcinoma of stomach (history of carcinoma of cervix—well treated)	—	Spindle-cell lipofibrosarcoma	1

Localization of Activity in Relation to Particle Size of Antigen

When the differential centrifugation procedures described under "methods" were used the following results pertaining to the sensitivity and specificity of the different fractions were obtained.

Sediment I had no antigenicity, while the final supernatant (III) had very good antigenicity but poor specificity. Supernatant I possessed relatively poor antigenicity but good specificity. Both Sediments II and III had very good antigenicity, but Sediment II had the better specificity. Furthermore, in contrast to Sediment III, Sediment II did not lose its antigenicity on storage in the deep freeze at -20°C .

Variables Introduced by Different Methods of Treatment of Serum

We used 114 sera from cases of untreated carcinoma and those with other diseases. Three cases of carcinoma receiving previous treatment were excluded. The sera were given numbers unknown to the person operating the test. The results of these tests, shown in Table V, indicate the importance of controlling certain variables in the treatment of sera to be tested, to ensure accuracy of results and to avoid false-positive or false-negative reactions.

It is apparent from these experiments that two factors are of paramount importance in determining the incidence of false positives and false negatives: (1) The particle size of antigen used in the sensitization of guinea-pigs. (2) The type of treatment which the serum undergoes—mainly storage period at 4°C ., and -20°C ., where antigenicity may be partially lost and where a non-specific pressor substance, possibly resulting from slow disintegration of the platelets, may be formed.* This pressor substance, due to platelet destruction, was further proved in other experiments to be described elsewhere. Storage at -40°C ., did not result in a loss of antigenicity nor in the production of false-positive reactions for as long as 150 days. Routinely, as mentioned before, the serum was left for one day at 4°C ., and then either tested or kept at -40°C ., and then tested.

Excluding Group I, where large-sized particles were used, it will be seen that the percentage of correct diagnosis was inversely proportional to the period of storage of serum at 4°C ., and at -20°C ., before testing.

Another experiment was done to compare the incidence of false-positive reactions in routinely stored sera left overnight in the ice chest at 4°C ., which would be comparable to the other group studied and referred to under "sensitivity and specificity of test as related to immunizing antigen."

*At the M. D. Anderson Hospital and Tumor Institute we have been using routinely drawn blood in heparinized syringes into lustrous tubes and have since rarely noticed this pressor effect.

Forty-seven sera from patients with other than neoplastic diseases were obtained from the chemistry laboratory of the Johns Hopkins Hospital. These were kept overnight at 40°C ., in the same way as the routinely stored sera, and then tested for non-specific pressor effect on non-sensitized uterine horns. Two out of 47 gave false-positive reactions, an incidence of 4.2%.

Reproducibility of Test

Platelet-intact plasmas from three Group O normal blood donors were added together to make a negative carcinoma pool, while platelet-intact plasmas from three cases with untreated carcinoma were also added together to make a positive carcinoma pool. Plasma was then pipetted in 2-ml. amounts into 50 sterile test-tubes and given numbers unknown to the person doing the test. Seven were from the carcinoma-positive pool and the other 43 were from the normal Group O pool: all of the positives and all of the negatives were correctly diagnosed.

Reproducibility of this test was also studied in another way. Sera from 15 untreated carcinoma cases were tested by "active sensitization" to antigens A and B repeatedly for a total of 35 times. In the interval after testing, these sera were kept frozen at -40°C .. In all 35 tests a positive reaction was obtained. Similarly, 3 sera from 31 untreated carcinoma cases were studied by "active sensitization" to antigen C for a total of 77 times over a period of two months of storage at -40°C .. In 75 of the 77 tests the reaction was positive.

A case of cancer of the lung shows this point. The test done on the patient's serum stored at -40°C ., showed consistently positive results when tested 11 times during a period of two months and compared very favourably with a fresh sample of serum obtained two months later and tested four times. However, this stored serum when tested on the tenth and eleventh time, although still positive, was getting weaker as compared with the previous tests and also with tests on the fresh serum. It was therefore possible to keep in this way a positive serum control from a carcinoma patient ready at all times for testing.

Discussion

The utilization of immunological techniques for diagnostic purposes has been limited in the past to detection of antibodies, a procedure inherited from the earlier days of bacteriology that has proved very fruitful in many bacterial diseases, but less so in parasitic and neoplastic diseases, and in many diseases of viral origin. The detection of antibodies necessitates that the host, in response to the presence of antigens, react by forming detectable antibodies in all cases, a step which may or may not take place in all patients, and therefore may account for the failures in the past attempts to detect antibodies constantly in animals or in patients with cancer. By looking for antigens instead of antibodies this difficulty is removed.

The problem of how best to detect these soluble antigens in the blood presents a number of difficulties. With the immunological techniques as normally employed, such as precipitation, agglutination, or complement fixation, univalent antibodies—that is, those antibodies with but one reactive group per antibody molecule—are not detectable. It has been found that an antibody which reacts demonstrably but does not precipitate may be present in some sera and that such antibody might be able to sensitize (Boyd, 1947). Furthermore, differences in the ability of antibodies of different species to fix complement or to sensitize guinea-pigs have been described by Kabat and Mayer (1948). Kabat and Landow (1942) have reported that an isolated guinea-pig uterus will contract even though containing amounts of antibody nitrogen not exceeding $0.01\text{ }\mu\text{g}$. This reaction is therefore much more sensitive than any *in vitro* immunological method.

These considerations make the Schultz-Dale the test of choice for detection of antigens, not only for its technical soundness as a specific immunological test but also for its great sensitivity.

TABLE V.—Variables Introduced by Different Methods of Treatment of Serum and by Particle Size of Antigen

Unknown Group No.	Treatment of Serum Storage in Days			Type of Sensitization	Total No. Sera Tested	Result of Test					Comments
	+Clot 4° C.	-Clot 4° C.	-Clot -20° C.			Correct Pos.	Correct Neg.	False Pos.	False Neg.	Correct	
I	1	—	5	Passive	25	6/11	6/14	8/14	5/11	48% (12/25)	Use of large-sized particle antigen blamed for failure
II	1	30	—	Active	18	4/9	1/5	4/5	5/9	36% (5/14)	Prolonged storage of serum at 4° C. blamed for failure
III	4	10	25	"	38	11/19	11/19	8/19	8/19	58% (22/38)	Prolonged storage at 4° C. and -20° C. blamed for failure
IV	1	2	—	"	16	7/7	7/9	2/9	0/7	88% (14/16)	Result good, but use of inert antigen cut down on specificity
V	1	7	—	"	17	8/8	8/9	1/9	0/9	94% (16/17)	Results excellent, but tests all weak owing to partial loss of antigenicity from storage at 4° C.

The results of tests described here on 707 sera from patients at Johns Hopkins and Baltimore City Hospitals show the value of this approach in the detection of a soluble antigen from the blood of patients with carcinoma.

The results obtained when the test is done under the best conditions yet developed shows a very high specificity of 96.8%, with a false positivity of 4.7 to 5% in cases with other diseases or persons coming to the Cancer Detection Clinic respectively. These cases have not been followed long enough to rule out the possibility of their having real positive reactions and may not represent false positives in the real sense. These figures are still in conformity with the requirements as put up by Dunn and Greenhouse (1951).

It should be emphasized here that this antigen is a common antigen for all carcinomas, as evidenced by the fact that it is present in carcinomatous tissues and in bloods from untreated carcinoma patients regardless of site of origin and type of carcinoma. This fact adds a lot to the practical usefulness of such an approach as a diagnostic procedure. Moreover, it should also be emphasized that this antigen present in carcinomas does not seem to show common antigenicity with sarcomas, Hodgkin's disease, leukaemia, or benign tumours studied. It has, however, common antigenic components with two of the three melanomas and with the one seminoma case studied.

This test, however, like many biological tests, has a number of pitfalls which should be carefully avoided if the best results are to be obtained. As already shown, the best antigen to be used for immunization should include only those particles of a small size. The best of these seem to be Sediment II and Sediment III. Inclusion of large-sized particles or the remainder of the supernatant increases false-positive reactions. Furthermore, it is best to use heparinized plasma drawn the same day or serum after 24 hours' storage at -4° C.

Passive as well as active sensitization could be used. Passive sensitization might provide a much more practical way, since serum with high antibody content could be lyophilized and kept available in the laboratory for such testing at any time. By use of the passive sensitization technique, methods might be developed for standardization of antibody titre in the guinea-pig or rabbit serum, and consequently that coating the uterine horn.

Of great interest are the results of the nine cases with a questionable clinical diagnosis of cancer, which were followed up to one year for pathological diagnosis, and which revealed the high specificity and accuracy of the test. This was apparent not only in the elimination of other tumours, such as sarcomas, and other conditions, such as lupus and fat necrosis, but also in the early detection of positive cancer cases in a period varying from 4 to 151 days before definite clinical and pathological diagnosis was made. Although these cases are too few to draw any conclusions from, they point to a very fundamental issue, which will be evaluated in subsequent work.

G. M., a case of squamous-cell carcinoma of the gums with invasion of the mandible, illustrates this point. She was treated by resection during the first operation on September 30, 1952, when a definite diagnosis of squamous-cell carcinoma, grade I, was made. The test was made on

February 1, 1953, and was positive. A second operation was done on February 26, 1953. The surgeon thought then that all the cancer tissue had been previously removed by composite resection of the lesion of the cheek, and during this time skin grafts were done. The patient became progressively worse, however, with very extensive recurrent carcinoma, involving the whole left side of the face, with multiple draining sinuses, and died on August 9, 1953. It is to be noted that the test was positive about a month before the second operation, when the patient was thought, on clinical grounds, to be free of carcinoma. Six months after the positive test, and five months after she was pronounced clinically free, the patient died with extensive carcinoma.

Summary

The detection of a specific soluble antigen in the sera of carcinoma patients, by use of the Schultz-Dale test on actively or passively sensitized uterine horns, is described. Under the best conditions yet found—that is, when the serum or plasma is used fresh or after storage at -40° C., or where platelet-intact or platelet-free plasma is used, and when the sensitizing antigen is of small particle size—this test shows a high degree of specificity and sensitivity, it being positive in 96.8% of carcinoma cases, in 4.8% of other diseases, and in 4 to 5% among cases from the Cancer Detection Clinic.

Further work is in progress at the University of Texas, M. D. Anderson Hospital and Tumor Institute, to help standardize this test and to evaluate on a larger scale the possibility of its use as an early diagnostic aid in individual cases and in the screening of carcinoma in the general population.

I wish to thank Dr. George S. Mirick, Associate Professor of Medicine at Johns Hopkins Hospital, for his advice and help and for making available the necessary laboratory facilities; Dr. George O. Gey for making available the HeLa strain from his tissue-culture laboratories; Dr. Bernard C. Brack for permission to obtain blood from patients coming to the Cancer Detection Clinic; Drs. E. Staten Wynne and R. W. Cumley for help in editing the manuscript; and Mrs. Marie Maselbas and Mr. John Lockner for valuable technical assistance.

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