

THE SHAW-MACKENZIE TEST FOR CANCER¹.

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THE work here described deals with 200 sera which have been examined by the Shaw-Mackenzie test for cancer. This has been purely in the nature of a preliminary investigation, and does not attempt any systematic study. The number of sera examined is, of course, much too small to allow of satisfactory statistical treatment, as there are too few cases of each type of disease.

TECHNIQUE.

As this has differed in several points from that used by Shaw-Mackenzie (1922, 1925) it will be described in detail.

Preparation of the carcinoma extract.

The malignant tissue to be used should be freed from fat and obviously normal tissue as much as possible, finely minced, and extracted in a Soxhlet apparatus with alcohol, ether and chloroform successively, for 12 hours each. The mixed extracts are then evaporated to dryness, leaving a semi-solid, sticky residue. Most of the extracted material is found in the alcoholic portion, but different samples of tissue vary in this respect. The residue is now weighed and saponified, using either caustic soda or potash—the particular base used does not seem to make any difference to the results of the test. The exact composition of the carcinoma extract is not known, but an amount of sodium hydrate is taken which is equivalent to the proportion of sodium required to combine with that weight of stearic acid. This generally proves satisfactory, but on one occasion (an extract from a cellular carcinoma) it was too much, and on another occasion (an extract of an epithelioma of the wrist), it was not nearly enough, and three times the theoretical amount of NaOH had to be added before saponification was complete. The extract and the required amount of an alcoholic solution of soda or potash is heated on a water bath with constant stirring; when the alcohol has evaporated, a few more c.c. are added. This is repeated two or three times, and the extract is then dried in a vacuum desiccator. It is of a firm consistency, and breaks with a smooth fracture. It keeps indefinitely if stored in small sealed tubes.

A stock solution of 1 in 1000 is made from the dry saponified extract by rubbing up with distilled water, and is sterilised and kept in the ice chest. A fresh supply should be made about once a month. A 1 in 5000 dilution is

¹ *The investigation upon which this report is based was carried out at the instance of the Departmental Committee on Cancer, Ministry of Health and of the Medical Research Council.*

the strength generally used for the actual test, and this should be made afresh from the stock solution each day. The pH of the stock solution should be adjusted to 7.4. Inattention to this point leads to the appearance of a precipitate in the extract (which however disappears on warming to 37° C.) and also causes a number of doubtful results. The more carefully the pH is adjusted the more definite seem to be the results of the test.

The final 1 in 5000 extract is very markedly opalescent.

Preparation of the cholin hydrochloride solution.

A 1 per cent. solution in distilled water should be made *each day*, even one night in the ice chest rendering it unreliable.

Collection of blood.

The serum used must be fresh. If possible the blood should be taken on the day that the test is to be made, and in no case should the test be postponed more than 24 hours after the taking of the blood. Sera older than this almost always give negative results. The blood should be collected before breakfast, or not sooner than four hours after a meal. The serum must not be allowed to come in contact with rubber, a glass syringe providing the best means for the collection.

Technique of the test.

Either the cancer extract or the solution of cholin hydrochloride may be used for the test. The technique is the same for both.

Small tubes should be used (about 2 × 3/16 in. is a very convenient size), stoppered with good quality ordinary corks, or by means of tight plugs of cotton wool. About 0.3 c.c. of serum and an equal quantity of extract are used for each test. The serum is first placed in the tube, and the extract layered on the top. Mixing of the two fluids must be avoided, and if such should accidentally occur a fresh tube must be prepared, as when the fluids are so mixed negative results are always obtained.

Shaw-Mackenzie lays stress on the accurate measuring of the serum and extract, but so long as approximately equal quantities of serum and test solution are used, there does not appear to be any special advantage to be gained from accurate measuring. It is convenient to use small pipettes made from glass tubing, with capillary points, and graduated to contain approximately 0.3 c.c. The point of such a pipette can be lowered well into the narrow tubes generally used for the test, and mixing of the fluids is thus avoided.

The tubes containing the sera and extract are incubated at 37° C. for 18 hours. It is an advantage to leave the tubes standing at room temperature for one hour before attempting to read the results. Many tubes which when first taken out of the incubator are "doubtful" become quite definitely either positive or negative when they have settled down to room temperature.

The marked opalescence of the carcinoma extract has been mentioned. In the case of normal sera (by "normal" here is meant sera from a subject in

normal health, and not merely non-cancerous) this opalescence disappears after incubation, leaving a colourless water-clear solution above the serum. In some non-cancerous, but pathological, sera this opalescence does not entirely disappear, the top of the extract still retaining it, though the lower part nearer the serum is clear. In some few instances the opalescence does not clear at all, and this occurs especially in the case of sera from cancer patients.

The reading of the results after incubation has presented some difficulties. If attention is paid to the pH of the extract, as explained above, and no mixing of the two fluids is allowed to occur, a positive reaction consists of a white ring appearing at the junction of the fluids. This ring may be very marked or only slight. In the case of negative sera there is no such ring, but many cases give indefinite results, a diffuse haziness appearing instead of the compact white ring. Very few sera from carcinoma patients have given this "doubtful" or "pseudopositive" reading, but the majority of such sera have been from non-cancerous patients. When the opalescence of the extract does not entirely clear after incubation, the cloudiness remaining at the top of the tube must not be mistaken for a ring occurring at the junction of the fluids.

Cloudy sera.

As is well known, blood taken after a meal yields cloudy serum due to the presence of fat. Such serum is quite unfit for the application of the Shaw-Mackenzie test, as owing to the initial cloudiness it is impossible to read the results after incubation, and a large percentage of wrong "positives" are likely to ensue. Such serum incubated for two or three hours with the co-enzyme of pancreatic lipase becomes clear, and is then suitable for testing. 0.3 c.c. of the co-enzyme is put up with 0.3 c.c. of serum and incubated for a few hours. The carcinoma extract is then added in the usual way.

This treatment with co-enzyme certainly clears up the milkiness due to a recent meal in the serum from a "normal" individual, but proved to be unreliable when applied to pathological sera. Shaw-Mackenzie states that the "addition of the co-enzyme to carcinoma cloudy serum does not completely effect clearance." This is true, but would matter little if it were only so in the case of carcinoma sera, but unfortunately it very frequently fails to clear up initial turbidity in many sera from other pathological conditions. If the initial turbidity fails to clear up, a wrongly "positive" result follows. From Table IV it will be seen that of fifteen non-cancerous cases where co-enzyme was used, only three gave correct results. These three sera completely cleared with co-enzyme, whereas the others did not. Possibly this cloudiness is not due to the same cause as that in normal sera after a meal. Indeed, the blood of thirteen of these cases was taken some 12 hours after the last meal, thus excluding milkiness due to recent food. It is thus evident that, unless the co-enzyme really has obviously cleared the serum, a positive result should be discounted.

*Test for Cancer**Haemoglobin-stained sera.*

Positive results are only rarely obtained with these, and such sera should not be used.

RESULTS OF TEST.

The results obtained are set forth in the following tables. No attempt has been made to state the percentage of correct results for the different types of disease, as an insufficient number of cases have been tried to admit of this treatment.

Table I. *Malignant growths.*

Type of case	No. of cases	+	-	?
Carcinoma of breast	15	10	4	1
„ rectum and colon	10	6	1	3
„ stomach	9	7	2	—
„ cervix uteri	8	8	—	—
„ tongue and floor of mouth	6	1	4	1
Malignant papilloma of bladder	4	4	—	—
Carcinoma of oesophagus	3	2	1	—
Sarcoma	3	2	1	—
Carcinoma of pancreas	1	1	—	—
„ lung	1	1	—	—
„ gall-bladder	1	1	—	—
„ ovary	1	1	—	—
„ prostate	1	1	—	—
„ cheek	1	1	—	—
„ parotid	1	1	—	—
„ larynx	1	1	—	—
„ thyroid	1	—	1	—
Epithelioma of pharynx	1	—	1	—
„ hand	1	—	1	—
„ ear	1	—	1	—
Myeloma of tibia	1	1	—	—
Total	71	49	17	5

Table II. *Benign or non-specified growths.*

Type	No. of cases	+	-	?
Fibro-adenoma of breast	1	—	1	—
Tumour of kidney	1	—	1	—
? Carcinoma of kidney	1	1	—	—
Papilloma of bladder (innocent)	1	—	1	—
Cerebral tumour. ? Sarcoma	1	1	—	—
Spinal tumour	1	1	—	—
Mediastinal tumour	1	—	1	—
Total	7	3	4	0

Table III. *Non-cancerous cases.*

Diagnosis	No. of cases	+	-	?
Gastric and duodenal ulcers	13	2	10	1
Appendicitis	8	1	5	2
Syphilis	8	2	4	2
Nephritis	7	—	4	3
Gall-bladder affections	5	1	3	1
Gonorrhoea	4	2	2	—
Ulcers, arm and leg	4	2	1	1
Myelocytic leukaemia	3	1	2	—

Table III—*continued*.

Diagnosis	No. of cases	+	-	?
Pulmonary tuberculosis	3	—	1	2
Tubercular peritonitis	2	—	2	—
Cirrhosis of liver	2	—	2	—
Pernicious anaemia	2	—	2	—
Renal calculus	2	—	1	1
Arthritis	2	—	2	—
Diabetes	2	—	2	—
Trauma	2	—	2	—
Hysteria	2	—	2	—
Auricular fibrillation	2	1	1	—
Chronic mastitis	1	1	—	—
Varicose veins	1	—	1	—
Exophthalmic goitre	1	1	—	—
Salpingitis	1	—	1	—
Addison's disease	1	—	1	—
Spleno-medullary leukaemia	1	—	1	—
Splenomegaly	1	—	—	1
Chronic myocardial failure	1	—	—	1
Urethral stricture	1	1	—	—
Diverticulitis	1	—	1	—
Dilated stomach	1	1	—	—
Duodenal dyspepsia	1	—	1	—
Rheumatic fever	1	—	1	—
Fistula	1	—	1	—
Hernia	1	—	1	—
Asthma	1	—	1	—
Osteitis fibrosa	1	—	—	1
Polioencephalitis	1	—	1	—
Encephalitis lethargica	1	—	1	—
Peripheral neuritis	1	—	—	1
Varicocele	1	—	1	—
Hodgkin's disease	1	—	1	—
Menstruation	5	2	2	1
Pregnancy	2	—	1	1
Total	102	18	65	19

Table IV. *Sera with added co-enzyme.*

Diagnosis	No. of cases	+	-	?
Carcinoma of breast	2	2	—	—
" stomach	1	1	—	—
" rectum and colon	2	2	—	—
Papilloma of bladder (benign)	1	1	—	—
Gastric and duodenal ulcer	2	—	1	1
Appendicitis	2	2	—	—
Pulmonary tuberculosis	2	—	—	2
Syphilis	3	3	—	—
Disseminated sclerosis	1	1	—	—
Diabetes	2	2	—	—
Pregnancy	2	—	2	—
Total	20	14	3	3

DISCUSSION OF CASES.

Malignant growths.

Four cases of carcinoma of the breast were negative. Of these, one patient had received radium; a second, radium and lead; a third, deep X-ray therapy; while the fourth was a case of early carcinoma of the breast (con-

firmed histologically). The patient was in good health in spite of the growth. Old serum was unavoidably used for one test, and this gave a doubtful result.

Three cases of carcinoma of the rectum gave doubtful results, but two of these were among the very earliest sera tested when no particular attention was being paid to pH. The third doubtful serum was that of an old person with a very advanced inoperable carcinoma of the colon. No details were obtainable for the case which gave a negative result.

The two cases of carcinoma which gave negative results were not confirmed histologically. One was a man of 70 with advanced inoperable carcinoma.

The case of carcinoma of the oesophagus which was negative was again a man aged 75.

Epitheliomata of hand, ear, etc., and carcinoma of the tongue and floor of the mouth, are almost invariably negative with this test. The only serum which gave a positive result in the present series was from a case having severe involvement of the glands of the neck.

A single case of myeloma which was positive is included in the list of malignant growths, though the malignancy of this type of growth is sometimes disputed.

Carcinomata of the cervix uteri gave strongly positive results in every instance, though these are epitheliomata. There is, however, a great systemic disturbance with these, whereas skin epitheliomata are seldom accompanied by any marked disorder of the patient's general health.

It should be noticed that wrong "negatives" occur in two types of case—those of advanced age and in a very serious condition, and those who have been receiving lead, radium or X-rays.

Benign or non-specified growths.

The fibro-adenoma of the breast, and the benign papilloma gave negative results, but the exact diagnoses of the other cases are not known.

Non-cancerous cases.

A case of Hodgkin's disease, which was negative, is included in this section, though this condition is regarded by many as one of malignancy.

One of the most important groups is that comprising gastric and duodenal ulcers. These are just the cases where doubt concerning possible malignancy is frequently felt by the clinician. Ten out of the thirteen cases gave entirely negative results. Two, however, were positive. In one instance the sample of blood was taken about twelve days after operation for acute symptoms due to perforation and the patient was still febrile, though, as will be seen later, fever does not necessarily lead to incorrect "positives." The other case was a patient who had had symptoms for 15 years; carcinoma was strongly suspected, but never proven.

Chronic appendicitis is another important class. Of eight cases, one was

definitely positive and two were pseudopositive. The former was an acute appendix abscess with high fever and so also was one of those giving a pseudopositive result, but the other was a sub-acute case in a child, of two months' duration. This serum was slightly cloudy, and should have had co-enzyme added in the hope of clearing it. Wrong "positives" obtained in sudden acute cases are relatively unimportant. The condition of these patients is generally obvious and the question of malignancy must seldom, if ever, be under consideration.

The group of gall-bladder cases was less satisfactory, as of five cases three only were negative. In one of these high fever was present, whereas it was absent from the case giving the positive.

Shaw-Mackenzie mentions having once obtained a positive result in a case of nephritis, and during the present investigation out of seven examined three gave doubtful or pseudopositive results.

Greater difficulties were experienced in syphilis and gonorrhoea. Of the former, out of eight cases, two were definitely positive, and two were doubtful. The sera which were negative or doubtful to the Shaw-Mackenzie test, also gave negative Wassermann reactions, whereas the Wassermann reaction was positive in both cases giving positives with the cancer extract. Two cases of gonorrhoea also gave positive results, but both sera were distinctly cloudy after incubation. All these sera were from out-patients, and blood could not be taken in any special time relation to the last meal. Consequently, practically none of the sera were entirely clear, and as has been pointed out before this leads to a large percentage error in the results.

In connection with cloudy sera, diabetes has been interesting. Shaw-Mackenzie finally decided that owing to this fact diabetic serum could not be distinguished from cancerous. In the present series only four examples were examined. Two of these were typically milky, and consequently gave strong positive reactions after incubation with co-enzyme and subsequent addition of cancer extract; the other two sera, however, happened to be quite clear, and both these were completely negative to the test. In one of these patients, the clinical condition was complicated by the presence of pulmonary tuberculosis, and in the other by a septic condition of the arm.

Tubercular cases are an important group. Of the five patients with pulmonary tuberculosis from whom sera was obtained, only one was completely negative. Two sera had to have co-enzyme added, but even so they did not entirely clear, and the results were consequently doubtful. Two of the others also gave doubtful results. The whole serum seemed to turn cloudy and hazy, and it was very difficult to decide how to read the test. On the other hand, two cases of tubercular peritonitis gave obviously negative results, though both cases were febrile, and of fairly long standing. Tuberculosis seems to be the greatest trouble in all the numerous cancer tests which have lately come to the fore, and Shaw-Mackenzie himself persistently found his test positive in tuberculosis.

A disappointing group was four cases of ulcer, of which two were trophic ulcers of the leg; one, varicose ulcers; and one, a large traumatic ulcer of the arm. Only the latter case was negative, the trophic ulcers both giving definitely positive sera. Such ulcers as these are frequently pre-cancerous, but so also are gastric ones, which, as we have seen, have (at any rate in the present series) yielded good results.

A few instances of heart conditions gave unexpected results. One case of auricular fibrillation was definitely and strongly positive, and though the other one was negative, yet the opalescence in the carcinoma extract did not clear in the least. The case of chronic myocardial failure had a slightly cloudy serum, which may have accounted for the doubtful result.

Pregnancy is another condition giving bad results with most cancer tests. Four cases (of about the seventh month) were tried, and of these three were negative and one was doubtful. Menstruation was also interesting. Five women were examined the day preceding menstruation, and two of them gave definite positives with the test, while a third was doubtful. Four of these women were examined on a later occasion, and each gave the same result as she had done before. A positive in this condition is not surprising, as almost every constituent of the blood is disturbed, and the only safe rule is never to take blood for examination at that time.

CHOLIN HYDROCHLORIDE SOLUTION.

This was used in 157 of the cases already described, in addition to the cancer extract. In 143 instances the results were identical with those obtained with the cancer extract. The remaining 14 are compared in Table V. Thus some cases were correct with the carcinoma extract and not with the cholin hydrochloride, and *vice versa*. The technique when using the cholin has the advantage of extreme simplicity.

Table V. *Comparison of cases which differ in reaction with carcinoma extract and cholin hydrochloride.*

Case no.	Diagnosis	Carcinoma	Ch. HCl.	Notes
9	Carcinoma of pancreas	++	-	—
22	Carcinoma of oesophagus	+	?	This was really only a difference in degree, the positive with carcinoma extract being only very slight.
23	Papilloma of bladder	+	-	The cholin solution mixed rather badly with the serum.
59	Duodenal ulcer	+	+	Very slight positive.
61	Gastric ulcer	+	-	A ? carcinoma case.
101	Myeloma	+	-	—
102	Bladder carcinoma	+	?	—
122	Thyroid carcinoma	-	+	Confirmed by section
143	Syphilis	+	-	Wassermann reaction ++
164	Gastric ulcer	++	-	—
206	Carcinoma of floor of mouth	?	-	—
184	Cholecystitis (acute)	++	+	Very slight reaction with cholin
214	Trophic ulcers	+	+	” — ”
225	Myelogenous leukaemia	++	-	—

GENERAL DISCUSSION OF RESULTS.

The lipolytic activity and anti-tryptic power of the serum have not been systematically investigated during the present work. Neither the reduced lipolytic nor the increased anti-tryptic power of the serum is specific for carcinoma cases, but combined with the Shaw-Mackenzie test they are undoubtedly useful. For instance, during menstruation, though the Shaw-Mackenzie test may be positive, the lipolytic activity of the serum is increased, instead of reduced as in carcinoma. Again, nephritic serum, which often gives a positive Shaw-Mackenzie test, has normal anti-tryptic properties, whereas in cancer this reaction is increased.

It is not yet known to what a positive Shaw-Mackenzie reaction is due. The majority of cancer tests seem to depend upon the total amount of protein present in the serum, or upon the ratio of albumin to globulin. Evidently the present test does not depend upon any enzyme action of the extract, as all enzymes must be destroyed during its preparation. If it depends upon the amount of protein in the serum, it is difficult to see why shaking, contact with rubber, standing under sterile conditions, etc., should change a "positive" to a "negative." Disappearance of opalescence in the antigen when incubated with normal serum, and imperfect, or non-disappearance of this opalescence in the case of cancerous and some other pathological sera, suggests at first the presence of a factor in normal sera which is absent in the others. But the fact that cancer sera lose their power of reaction under certain conditions, suggests a different explanation. Cancer sera (and some other pathological sera) may have some inhibiting factor present, which is absent in normal sera, and which is destroyed by shaking, etc.

This test compares favourably with other cancer tests. There are many interesting points of similarity between them. Thus Faludi (1925), using Botelho's test, found that it gave negative results in skin epitheliomata, and positives in tuberculosis, pregnancy, cirrhosis of the liver, and in hydraemia of renal or cardiac origin. Giauni (1925) found the same test positive in 35 per cent. of cases of tuberculosis. Volkmann (1926), using various modifications of Abderhalden's reaction, found that radiation treatment of the patient caused disturbance of the test. Epstein (1913), also with a modified Abderhalden test, found a negative result in an advanced case with extreme cachexia, and Thomas and Binetti (1922) had a similar experience with their own test. Using a complement-fixation test Dungern (1912) found it positive in tuberculosis and syphilis. These conditions also give positive reactions with Kahn's (1923) test, and Saxl and Kelen (1925) found that their trypsin flocculation test was given alike by tuberculosis, nephritis, pregnancy and uncompensated heart disease, and finally Vorschütz (1923) found similar serum reactions in pregnancy, carcinoma and tuberculosis. On the other hand, Fry (1925, 1926) had positive results with his test in skin epitheliomata, but a poor percentage of correct results in carcinoma of the rectum, which he

attributes to the local nature of the latter disease—an explanation which seems inadequate, as skin growths are just as local and even less likely to give rise to systemic disturbances.

The Shaw-Mackenzie test, though not specific for cancer, would probably prove useful in many doubtful cases. It is sufficiently simple in regard to technique, especially the use of cholin hydrochloride. Many of the pathological conditions which have been investigated are unimportant from the point of view of the practical usefulness of the test. In conditions where the clinician is most likely to require the help of a blood test, such as gastric and duodenal ulcer, chronic appendicitis, tubercular peritonitis, the results were very good.

The writer has been frequently asked whether the test shows the presence of new growths in their early stages, or of pre-cancerous conditions. The great difficulty of determining this point does not seem to be appreciated. A growth in its very early stages is usually not diagnosed as such, and as to whether a condition is pre-cancerous or not can only be determined by keeping in touch with the patient for some time, possibly years. In addition, the pre-cancerous state may improve or disappear as the result of treatment, when obviously it would be impossible to say whether it had ever been pre-cancerous. Some of the patients in the present series who have given wrong "positive" or doubtful results may well be in a pre-cancerous condition, but in the meantime one is obliged to put such cases in the category of incorrect results.

SUMMARY OF RESULTS.

(1) Of 71 cases of malignant growths the Shaw-Mackenzie test was positive in 49, doubtful in 5 and negative in 17. Epitheliomata of the skin all gave negative results, and some cases which had been treated with lead, X-rays or radium, and some cases of advanced inoperable carcinoma in old people also gave negatives.

(2) In 102 cases of various diseases other than growths (malignant or otherwise) negatives were obtained in 65 cases while 19 were doubtful and 18 were definitely positive. The chief conditions likely to give wrong "positives" are venereal disease, heart cases, nephritis, pulmonary tuberculosis, pregnancy and menstruation.

(3) Cloudy sera do not always clear on incubation with co-enzyme of pancreatic lipase, and unless such clearing takes place they should be discarded, as a high percentage of false positive results occurs in these instances.

(4) A 1 per cent. solution of cholin hydrochloride gave very similar results to the carcinoma extract.

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