cancer-producing properties after exposure to a temperature of 880°.

3. The cancer-producing substance present in coal tar is formed only in very small amounts below 450°; at 560° it appears in much larger quantity, and this increase continues at a slower rate up to 1,250°.

I wish to express my thanks to the staff of H.M. Fuel Research Station for their kindness in supplying the series of coal tars made at known temperatures.

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Remarks

ON

A NEW FLOCCULATION REACTION FOR THE SERO-DIAGNOSIS OF MALIGNANT DISEASE.

BY

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INTRODUCTION.

THE value of accurate diagnosis of malignant disease, especially in early or latent cases, by a serological method does not require emphasis. Many attempts by various methods have been directed to this end, hitherto without conspicuous success, although the occurrence of profound changes, such as anaemia and cachexia, in the later stages of malignant disease would suggest the possibility of accompanying or antecedent changes in the serum.

Murray¹ has recently brought forward some evidence that a neoplastic reaction evoked at one site in an animal confers some resistance to such a change at another site, and he suggests that there is some systemic constitutional change. If such be the case, then it is not improbable that the medium of distribution of the locally produced change is the blood. On these grounds a serological reaction would appear *a priori* to be practicable.

In experiments carried out by me, it was found that extracts of cancer tissue used as antigen in complement fixation experiments often showed marked anti-complementary properties. It seemed possible that a method, in the nature of a colloidal flocculation reaction, which dispenses with the haemolytic system, might prove of value. Moreover, the fact that the Sachs-Georgi antigen employed in Professor Hirschfeld's laboratory, Warsaw,² gave a number of positive reactions with serums from cases of carcinoma, seemed to indicate the possibility of obtaining a specific flocculation reaction for malignant disease.

HISTORICAL.

The complement fixation test has been largely used to discover specific cancer antibodies in the serum. This method was first tested by Sampietro and Tesa in 1908,³ and they were followed by numerous other investigators, some with favourable and others with indefinite results. Weinberg and Mello⁴ obtained only twenty positive results from the serums of 100 cases of cancer. Subsequent observers also found the reaction to be without value. In 1912 von Dungern⁵ published the results of his method, for which he claimed accuracy in 100 per cent. of cases. Later observers have failed to substantiate his claims.

Other reactions have been described, such as the precipitin reaction, used by Engel in 1904, and the anaphylactic methods examined by Pfeiffer and Finsterlin.⁶ The haemolytic properties of cancer serum were noted by Weil⁷ and further examined by Crile.⁸ On this property was based the cuti-reaction of Elsberg.⁹

The antitryptic reaction described by Marcus¹⁰ was more fully examined by Brieger and Trebing.¹¹ Other reactions which may be mentioned are the meiotagmin reaction of Ascoli and Izar,¹² the ferment reaction of Abderhalden,¹³ the fallacies of which were pointed out by Leitch,¹⁴ and the "epiphanin" reaction of Weichardt.¹⁵ Freund and Kaminer¹⁶ have published a turbidity test based on the theory of a destructive action of normal serum on cancer cells. Shaw-Mackenzie¹⁷ describes a similar turbidity test for carcinoma, using saponified extracts of cancer tissue.

A chemical precipitation reaction is described by Kahn,¹⁸ to which class Botelho's reaction also belongs.¹⁹ The latter has been shown by me to have little diagnostic value.²⁰ The rate of sedimentation of red corpuscles has been used for the diagnosis of cancer by Hoffgaard.²¹

The flocculation method was first used by Weil and Braun.²² They noted the flocculating power of cancer serums upon lecithin solution in 17 cases. During the course of this work Roffo²³ has published a flocculation reaction for the diagnosis of malignant tumours based upon the Sachs-Georgi reaction, but the details of his method are not very definite.

METHODS.

1. Antigen.

In the endeavour to find a satisfactory antigen over two hundred preliminary experiments were carried out with cancer serums, using different extracts of tumour tissue. None of these antigens nor various combinations of them proved consistently satisfactory. No better results were given by a "polyvalent" antigen of several carcinomatous tissues, an ether extract after the method of Shaw-Mackenzie, or a saline extract. The following method has been finally adopted, and has proved in general satisfactory.

Method of Preparation.

Breast carcinomata, preferably of a rapidly growing cellular rather than of a scirrhous type, have been the tissues selected. The breast is received sterile from the operating theatre. A small portion is taken for microscopical examination. The breast is then frozen in the refrigerator at about -6° C. for three to five days. The growth can then be readily dissected out and cleaned from the hard surrounding fatty tissue.

The tissue is minced fine, with sterile precautions, and successive portions are frozen with liquid oxygen and pounded to a fine state of division in a sterile mortar while so frozen. This step is not essential. Carcinomatous tissues minced very finely (by means of a mincer made by the Laboratory Supply Association) and treated as described below have recently given equally satisfactory antigens. The tissue is weighed (for example, 10 grams) and double the weight of sterile saline is added (for example, 20 c.cm.) and the whole shaken and allowed to extract in the ice-chest overnight. The tissue extract is centrifuged down and the supernatant fluid pipetted off. The same volume of sterile saline is again added, and the procedure repeated four times till the supernatant fluid is free from colour. The saline extraction extends over a period of five days. The saline extracts are rejected and the washed tumour tissue is dried overnight at 37° C. on a porcelain dish. When dry the tissue is weighed, and of acetone five times the weight is added to the tissue, which is extracted in a well stoppered glass bottle in the cold incubator at 18 to 20° C. for seven days, with frequent shaking. At the end of this period the acetone is filtered off through filter-paper (first acetone extract). Half the original bulk of acetone is added and the extract replaced at 18 to 20° C. for twenty-four hours. The acetone is again filtered off (second acetone extract). These acetone extracts are rejected. The remaining tumour tissue is kept at 18° C. till dry, or at 37° C. for two hours, and ground up. Absolute alcohol equal to five times the weight of dried tissue is added. The tissue is extracted at 18 to 20° C. for ten days. The alcohol is filtered off through filter-paper into an amber-coloured glass-stoppered bottle. This forms the stock alcoholic antigen, which contains the saline-insoluble alcohol-soluble acetone-insoluble substances of the tumour tissue.

According to the strength of the antigen, the stock solution is diluted with absolute alcohol. This amount is determined for each antigen by finding the dilution which gives the maximum flocculation with positive serums. This appears usually to be 1 in 4, but varies considerably with different extracts.

2. Solutions Required.

(a) A 1 per cent. alcoholic solution of cholesterol is prepared by dissolving 1 gram of cholesterol (Kahlbaum) in 100 c.cm. of absolute alcohol in a glass-stoppered bottle.

(b) Sterile normal (0.85 per cent.) saline. The solution is made with distilled water, and should be free from calcium.

3. Apparatus.

(a) The trays, flocculation tubes, and pipettes are those used in the Sigma reaction.²¹ They are sterilized by dry heat.

(b) The dropping apparatus for preparing the saline emulsion of antigen is shown in the drawing (Fig. 1). It consists of a Winchester flask, tightly corked with a large rubber stopper, through which pass: (1) The delivery tube, to the outer end of which the end of a Dreyer-Ward dropping pipette is attached; the inner end is drawn out and lies below the level of the saline

FIG. 1.—Dropping apparatus for preparing saline emulsion of antigen.

in the Winchester. (2) A tube to which a Higginson's syringe is attached, for raising the air pressure inside the flask. (3) A mercury U-tube, which registers the air pressure inside the flask. The apparatus is very simple, and has been in constant use without attention for several years. Alternatively, the Dreyer-Ward dropping apparatus may be used.²⁴

(c) Two water-baths, one at 55° C. for inactivation of the serums, and an open one at 37° C. (kept at constant level) for incubation. A simple constant-level bath can be made by inverting a Winchester bottle full of water held in the rings of a retort-stand, so that the cork, which has a fairly wide hole through it, just meets the level of the water in the bath.

FIG. 2.

(d) An agglutinoscope for reading the results is useful. The one I have used consists of a wooden box, which contains a strip-light illuminating a slit in front of which is a tray with holes for the agglutination tubes.

4. Test Serums.

The serums were obtained by venipuncture at the bend of the elbow, with sterile precautions. The serum is allowed to stand in contact with the clot in the ice-chest overnight. It is then pipetted off, centrifuged free from red cells, and heated at 55° C. for half an hour. At first the serums were heated at 55° C. for one hour, but the shorter period has been found equally satisfactory.² The serums, after heating, are not tested for twenty-four hours, during which time they are kept in the ice-chest. Anomalous results were sometimes found if the serums were tested immediately after heating. This has also been found to be the case with the Sachs-Georgi reaction (Browning and Mackenzie²⁵).

Preparation of Saline Emulsion of Antigen.

Immediately before use 0.1 c.cm. of 1 per cent. cholesterolin (Kahlbaum) is added to each 1 c.cm. of diluted antigen; 1 c.cm. of cholesterolinized antigen is then placed in a graduated cylinder, and saline is delivered drop by drop from the dropping apparatus, from a height of 34 cm., at a rate giving a dilution of 1 in 13 in one and a half minutes. The antigen is always freshly prepared, and is translucent, faintly bluish and opalescent, and only slightly turbid. Only one dilution of antigen is used. Prepared in this manner the saline emulsions give the same degree of flocculation with a positive serum on successive days.

Arrangement of Test.

Only four flocculation tubes are used for each test serum; the same serum dilutions are put up as for the Sigma reaction, omitting only the first tube containing 20 drops of pure serum. The 1 in 20 dilutions of serum were at first put up in addition, but it was found that even normal serums sometimes showed flocculations in the last tube of this dilution, and that some serums tended to flocculate in the last two tubes. As a routine, therefore, four serum dilutions have been put up, containing 10, 5, 2, and 1 drops of serum respectively, and 0, 5, 8, and 9 drops of normal saline. To the four tubes are added 15 drops of the saline emulsion of antigen. A control tube containing 10 drops of saline and 15 drops of antigen is put up, together with positive and negative controls. The pipette is washed with distilled water, alcohol, and ether, and dried, after use for each test serum. The tubes are shaken in order from right to left, and are incubated for three hours at 37° C. in a constant-level water-bath. The water level is maintained at half the level of the fluid in the flocculation tubes. The trays are then stood overnight (about eighteen hours) at room temperature (about 18 to 22° C.).

Readings.

The tests are read after three hours, and again at the end of eighteen hours. With strong positive serums there is good flocculation at the end of three hours, which is much increased by the further period at room temperature. A weak flocculation, showing only traces at the end of three hours, is then easily visible. The flocculi are generally much coarser than in the Sigma reaction, and are visible with the naked eye without the use of a lens. They appear to be lighter, and do not sink to the bottom of the tube so readily as in the Sigma reaction.

"S++" indicates a degree of flocculation in which only a few large clumps remain in the fluid, or have sunk to the bottom of the tube. When there are coarse granular flocculi evenly distributed in the fluid and easily visible to the naked eye, this is taken as "S" reading. A "Tr" reading is only just visible to the naked eye. Turbidity less than this, visible only with a lens, may be neglected, as normal serums may give this degree of turbidity. Unless one of the four tubes shows an "S" reading the reaction is considered to be negative.

Source of Material.

The serums were obtained chiefly from patients undergoing either in-patient or out-patient treatment at this hospital. In the case of malignant disease the clinical diagnosis has, in the majority of cases, been controlled by operation, microscopical sections, or *post-mortem* examinations. In the absence of such positive evidence, where there was doubt about the correctness of the clinical diagnosis the cases have been considered separately.

The control serums have been obtained from normal healthy individuals, male and female, and from patients with various diseases other than malignant undergoing treatment in this hospital. I am also indebted for control serums to the Central Laboratory, Ministry of Pensions, obtained from patients free from evidence of cancer.

In order to obtain a just appreciation of the value of such a reaction the elimination of bias in the readings is of the utmost importance. To prevent such bias the serums were numbered, the tests were put up, and the readings made without knowledge of the source from which the serums were derived. Only then were the results compared with

the information as to the clinical characters of the case from which the serum was obtained. Doubtful border-line readings have been classed as incorrect.

RESULTS.

By this method 500 serums have been examined by three separate antigens. These antigens were prepared in a similar manner, but were derived from three separate sources. All were prepared from cases of carcinoma of the breast by the above method. The

FIG. 3.—Tube 1, Saline control: Saline 10 drops + antigen 15 drops. Tube 2: Serum 10 drops + antigen 15 drops (dilution 1 in 2.5). Reading, S+. Tube 3: Serum 5 drops + saline 5 drops + antigen 15 drops (dilution 1 in 5.2). Reading, S++. Tube 4: Serum 2 drops + saline 8 drops + antigen 15 drops (dilution 1 in 13). Reading, S+. Tube 5: Serum 1 drop + saline 9 drops + antigen 15 drops (dilution 1 in 26). Reading, S. Typical reaction with a cancer serum. Tubes 6, 7, 8, and 9, similar quantities. Completely negative reaction with a non-cancer serum.

first two were used undiluted and received only 0.05 c.cm. of 1 per cent. cholesterolin per 1 c.cm.; the third was diluted 1 in 4 with absolute alcohol and received 0.1 c.cm. of 1 per cent. cholesterolin per 1 c.cm.

The results obtained are as follows: In 500 cancer and control cases a correct result was obtained in 75 per cent. Out of 239 cases of malignant disease the result was correct in 170 (71 per cent.). In 261 controls of all kinds, comprising healthy individuals and others with various diseases, 204 were correct (78 per cent.). These results have been analysed according to the site or organ affected by the malignant process, to discover how the reaction is affected, and whether any light is thrown on it thereby.

Since the foregoing figures were compiled a further 125 serums have been examined by the third antigen, with correct results in 81 per cent. of cases of malignant disease, and 82 per cent. correct in the controls.

TABLE I.—*Malignant Disease.*

Site of Disease.	Number of Cases.	Number Correct.	Percentage Correct.	Evidence Accepted of Malignancy.		
				Clinical.	Post-mortem.	Microscopical.
A. CARCINOMA :						
Buccal cavity (excluding tongue)	29	20	70.0	18	—	6
Tongue	8	5	62.5	4	1	3
Oesophagus	5	4	80.0	2	3	3
Stomach	12	9	75.0	6	2	5
Colon	7	6	86.0	4	1	2
Rectum	35	19	54.0	10	6	25
Adrenals	2	1	50.0	—	2	2
Thyroid	1	1	100.0	1	—	1
Intra-abdominal (primary site unknown)	5	4	80.0	4	—	—
Bladder and genito-urinary (male)	14	12	86.0	9	1	4
Breast	50	29	58.0	12	—	36
Cervix uteri	39	34	87.5	19	2	14
Vulva and vagina ...	6	2	33.0	—	—	6
Skin (epithelioma head and neck)	9	9	100.0	4	—	5
	222	155	70.0	93	18	112
B. SARCOMA :						
Lung and thorax... ..	4	3	75.0	4	—	—
Bone—						
Pelvis	4	4	100.0	2	2	2
Femur	3	2	66.0	1	—	2
Foot	1	1	100.0	—	—	1
Lymphatic glands ...	2	2	100.0	2	—	—
	14	12	86.0	9	2	5
C. LYMPHADENOMA ...	3	3	100.0	—	1	3
Total	239	170	71.0	102	21	120

Carcinoma.

With all three antigens a high percentage of positive results is obtained with carcinoma of the alimentary tract. The high percentage shown by oesophageal (80 per cent.) and stomach growths (75 per cent.) is also noteworthy, as these are "inaccessible," and visible only by indirect methods. In cases of growths of the rectum positive results are much less numerous, and are amongst the lowest recorded (54 per cent.). This is rather striking, and suggests that it may be due to the local character of the growth at this site. The stage in which most of the rectal cases were seen was early, with little or no spread. This was determined at operation by Miles's abdomino-perineal method, and by subsequent examination of the parts removed. Where there were visceral deposits a positive result was often obtained.

Another striking feature is the low percentage of successes (58) given by breast cancers. This is all the more remarkable when one considers that all the antigens were derived from breast tissue. Here again it seems possible that the size and local character of the growth is the explanation, since a positive result was generally obtained if metastases were present.

These results contrast strongly with those obtained with carcinoma of the cervix and body of the uterus, in which the percentage of successes was strikingly high (87.5) and the flocculations were usually very well marked. The reason for this is evident, as most were advanced inoperable cases, though a few were operable. It may be mentioned that out of five negative reactions three were doubtful cases of carcinoma.

Bladder cases (86 per cent.) and epithelioma of the skin (100 per cent.), though not numerous, gave satisfactory results. In general, squamous-celled growths appear to react better than growths of glandular origin. Out of 222

cases of carcinoma of all sites and varieties 155 gave a correct result=70 per cent. correct.

Size of Growth.—With reference to what was said above concerning the size of the growth in the breast influencing the reaction, further evidence is afforded by rodent ulcers: out of four cases, three, which were of very small size, gave negative results, while the fourth was very extensive and gave a strong positive reaction.

Sarcoma.

The antigens reacted well with the serums of cases of sarcoma, the results showing that in 14 cases of all varieties, 12 gave a positive result=86 per cent. correct. Sarcomata at all sites reacted equally strongly. This may be due to the fact that the blood stream is more intimately connected with, and therefore affected by, the sarcomata.

Three cases of lymphadenoma (Hodgkin's disease) were investigated, and all three gave strong positive flocculations. If the lymphadenomata are classed among the neoplasms of the lymphatic system, with Mallory and the American school, this result may be regarded as correct; but if they are to be classed with the infective granulomata, the result would be incorrect. In any case, blood changes in the disease undoubtedly occur.

TABLE II.—*Controls.*

Type.	Number of Cases.	Number Correct.	Percentage Correct.	Evidence of Non-malignancy.		
				Clinical.	Post-mortem.	Microscopical.
1. Non-malignant tumours	19	13	68.5	5	—	14
2. Healthy individuals...	21	20	95.0	21	—	—
3. Syphilitic disease ...	15	11	73.0	15	—	—
4. Medical conditions ...	111	86	77.5	59	3	4
5. Surgical conditions (non-neoplastic)	95	74	78.0	62	1	28
Total	261	204	78.0	162	4	46

1. The non-malignant tumours, comprising papillomata, fibromyomata, adenomyomata, ovarian cysts, fibroadenomata, and an enchondroma, have been classed among the controls. The results obtained with them are rather difficult to assess. On the one hand, a few of the large tumours, such as fibroids of the uterus and adenomyomata, have given weak positive reactions. These have been classed as incorrect reactions. This accounts for the rather low figures of correct results (68.5 per cent.). A large suppurating ovarian cyst, discovered at operation, gave a strong positive reaction, as did a case of fibroids with an ovarian dermoid cyst. The papillomata, which were of small size, were uniformly negative.

2. Twenty-one healthy individuals gave completely negative results, usually without a trace of flocculation, except in the case of one female, whose serum gave a weak positive. A second sample of this serum at a later date gave a negative result. (As additional controls, serums from individuals suffering from various diseases other than neoplastic have been examined. These have been grouped for convenience into medical and surgical conditions.)

3. In view of the various flocculation reactions for syphilitic disease, the results obtained with the present reaction in the case of serums from syphilitics are of special interest, and have therefore been extracted from the general body of the results obtained in non-neoplastic diseases. It will be observed that in 15 cases of syphilitic disease of all stages except the primary, the reaction was negative in 11 (73 per cent. of cases). These cases were all free from evidence of new growth. Where, however, new growth was present in conjunction with syphilis, as in certain cases of carcinoma of cervix or tongue showing a positive Wassermann reaction, a positive reaction was obtained. It is evident, therefore, that the reaction is different in character from the flocculation reactions for syphilitic diseases, a subject which receives consideration below.

4. The heading "Medical conditions" includes alimentary disorders, cardiac and blood diseases, diseases of the ductless glands, skin lesions, tuberculosis and other pulmonary conditions, and, in addition, indefinite or trivial disorders in patients attending the out-patient department, serums sent from outside sources, and the like. All were from patients considered free from malignant disease. In the alimentary disorders, gastric ulcer and dyspepsia, a high percentage (90) of correct results was obtained. Active pulmonary tuberculosis or other tuberculous conditions gave a rather large number of false positives. Over the whole series of 111 cases, comprising a great variety of medical disorders, 77.5 per cent. of the cases gave correct negative results. Of the negative controls, 59 were definitely free from malignant disease on clinical grounds, 3 were verified by *post-mortem* and microscopical examination, and 1 by microscopical evidence only—a total of 63. Of these 63 definitely non-malignant cases negative results were obtained in 76 per cent.

5. Among the more frequent surgical diseases, gastric and duodenal ulcers, the majority of which were confirmed by operation, gave a good proportion of correct results (86 per cent.). Breast cases also showed 86 per cent. One case of surgical tuberculosis (spinal caries) gave a strong false positive. Surgical conditions show the same total percentage of correct results as medical conditions (78 per cent.). Of the above cases 90 were verified as non-malignant, either by operation or direct examination, 28 of these being confirmed microscopically. In 5 cases the evidence was lacking. Among the 90 verified cases, 73 gave a correct negative result (81 per cent.).

Evidence of Malignancy.

Of the 239 cases regarded as malignant, evidence of malignancy was definite in 222, or 93 per cent. Of these, microscopical examination was carried out in 120, or 50 per cent. of the cases, and *post-mortem* examination with microscopical diagnosis in 21. The clinical evidence was regarded as undoubted in 102. These were confirmed either by operation or by direct examination in the case of accessible growths. In these 222 definite cases of carcinoma, positive reactions were obtained in 158, or 71 per cent. correct. This is about the same percentage as is obtained by the Wassermann reaction in untreated cases of syphilis of all kinds.*

Evidence of Non-malignancy.

Of the 261 cases regarded as non-malignant 208 were verified, 162 upon clinical grounds, and 46 by microscopical and *post-mortem* evidence. Of these 208 cases, 164 gave a correct negative result (79 per cent.).

Incorrect Reactions.

A. *False negatives* were given by 69 of the 239 cases of malignant disease (28.8 per cent.). Of the 222 verified cases, 62 gave negative or doubtful reactions (28 per cent.).

B. *False Positives*.—Of 25 serums among the medical cases giving false positive reactions, no fewer than 14 (56 per cent.) were derived from outside sources, and of the 21 false positives* given by serums from surgical cases 4 were from outside sources—18 in all. Concerning these 18 serums no satisfactory information could be obtained, though they were all believed to be from non-malignant cases. The conditions under which the serums had remained after withdrawal of the blood, and the lapse of time after withdrawal, were unknown. These conditions may have militated against a correct result.

Of the remaining 28 medical and surgical cases, 10 showed only weak or doubtful positive reactions. These have been placed in the scale against the reaction.

Value of Reaction in Diagnosis.

In certain supposed malignant cases the flocculation reaction proved more accurate than the clinical diagnosis, for in no fewer than 34 cases its correctness was afterwards verified by operative, *post-mortem*, or microscopical examination. On the other hand, in 17 cases the reaction

supported the erroneous clinical diagnosis of malignant disease. In supposed non-malignant cases a positive reaction was obtained in 5 cases, which were subsequently proved to be malignant. On three occasions the reaction was incorrect.

Influence of Removal of Growth.

Removal of a growth, provided that metastases are absent, appears to cause a diminution or actual inhibition of flocculation in some cases, though this has not occurred in all. Thus, in one case of carcinoma of the breast which gave a positive flocculation prior to operation, the blood, when investigated six weeks later, gave only a weak positive reaction. A second case gave only a doubtful positive flocculation previous to operation. On microscopic examination the tumour showed changes suggestive of early malignant disease. The blood, when examined ten weeks later, gave a rather stronger reaction. A third examination seventeen weeks after operation gave a definite positive reaction. The patient, after operation, had received x-ray treatment. On the other hand, two patients who had had radical excisions of the breast, and had no signs of recurrence, the one fifteen months and the other four years after operation, both gave completely negative reactions.

Two cases of carcinoma of the rectum, on whom complete excisions by Miles's abdomino-perineal method had been performed, without signs of recurrence four and a half and five and a half years afterwards respectively, gave negative reactions. In these cases flocculation reactions had not been performed prior to operation. A case of carcinoma of the body of the uterus, which had been extensively treated by radium, gave nevertheless a strong positive reaction, due, no doubt, to the abdominal metastases present.

Relation to Wassermann Reaction.

In 487 out of the 500 cases Wassermann reactions were performed; two serums persistently proved to be anti-complementary. In all but 5 of the 239 cases of malignant disease Wassermann reactions were done, one serum being anti-complementary. Twenty-one positive Wassermann reactions were obtained among these 233 cases, including one case of lymphadenoma, in comparison with the 170 positive flocculations. On the 261 controls, 253 Wassermann reactions were done, one being anti-complementary. These gave 18 positive reactions. They were accompanied by only 7 positive flocculations. Among the latter was a case of fibroids and another of adenomyoma of the uterus. A third case, diagnosed as carcinoma of the uterus, proved to be chronic metritis.

It is evident from the above that the flocculation reaction here described is dependent upon factors different from those in the Wassermann reaction. The reacting substance in the serum is probably not euglobulin nor the globulin fraction upon which the Sachs-Georgi flocculation is said to depend (Browning and Mackenzie²⁵). The addition of an equal volume of a saturated solution of ammonium sulphate to the serum diminishes, but does not inhibit, the reaction. Further, the antigenic substances are almost certainly different. This is shown by the fact that carcinoma serums rarely if ever react positively with the Sigma antigen in the absence of a syphilitic factor, even if used in the same dilution and treated in the same manner as the antigen here described.

FACTORS INFLUENCING THE REACTION.

A. Serum.

1. *Heat*.—Positive serums do not flocculate unless they have been heated. No experiments have been done to show what is the least period of heating required for the reaction. Increase of time from a quarter of an hour to three hours does not appear appreciably to affect the result.

2. *Age of Serums*.—Serums appear to be unstable if the reaction is performed immediately after heating, and variable reactions may be obtained. Hence, they are not examined for twenty-four hours after heating. Within

* Since the compilation of these figures one of the cases included therein has returned to hospital with a well marked cancer of the oesophagus, verified by microscopical section.

limits, lapse of time after withdrawal of the blood does not seem to affect the reaction, positive serums giving approximately the same results on successive days. The reaction tends gradually to disappear. It is not known whether this occurs in sterile serums kept in sealed tubes.

3. *Infected Serums.*—Absolute sterility of the serums does not appear to be necessary, but gross infection causes anomalous results.

B. Antigen.

1. *Dilution.*—The antigen, unless diluted to a proper strength before the emulsion is made, may show spontaneous flocculation, or no flocculation may occur.

2. *Cholesterin.*—Flocculation does not take place, or is meagre, without the addition of cholesterin. The flocculation is not so marked with the smaller amounts of cholesterin.

3. *Saline.*—The use of distilled water instead of saline for making the emulsion does not seem to prevent flocculation.

C. Incubation.

Incubation at 37° C. for longer periods than three hours, up to twenty-four hours, does not increase the reaction. The reaction does not occur so readily at 55° C. If the trays are placed in the 37° C. incubator after the three hours in the water-bath the reaction does not proceed so far as when they are kept at room temperature after the water-bath.

DISCUSSION.

1. Nature of Reaction.

In common with other colloid flocculation tests—for example, Sigma and Sachs-Georgi—the precise nature of the reaction is not at all clear, nor are the substances upon which the reaction depends any better defined. It is evident that the reaction is due to a diminution of surface tension in an unstable colloidal system, in the course of which aggregates are formed of visible size. The nature of these colloids is unknown.

2. Specificity.

That this reaction is not at present absolutely specific for malignant disease is evident from the false positives shown above, notably with tuberculosis and certain febrile and other conditions, but the antigen has at least some specific relations with human serums.

(a) *Rat Antigen.*—An antigen prepared in a manner precisely similar to the human antigen, from a rat carcinoma, gave no flocculation at all with carcinoma serums which had already given flocculations with the human antigen. An antigen prepared from this rat carcinoma according to the formula of Roffo,²³ similarly gave no flocculation using the Sachs-Georgi method—a result opposed to those of Roffo. Moreover, cross-experiments with nine serums from rats grafted with rat carcinoma gave no flocculation with the human antigen, nor did they give any flocculation with the antigen prepared from their homologous tumour.

(b) *Lecithin-Cholesterin Antigen.*—An “artificial” antigen, prepared from a 1 per cent. solution of ovolecithin (B.D.H.), diluted with alcohol 1 in 4, to which 0.1 c.cm. of a 1 per cent. solution of cholesterin per 1 c.cm. was added in a manner similar to the carcinoma antigen, did not react in the same way with carcinoma serums, although it showed in some cases flocculation with such serums. It seems evident, therefore, that the carcinoma antigen has some specific properties.

(c) *Cellular Disintegration.*—The non-specific reactions which the carcinoma antigen exhibited seemed to be related to some extent to cellular disintegration. Thus, in the tuberculous cases there was considerable tissue or bony destruction. In the case of metritis, there was much cellular destruction and pus formation. Possibly the reaction may show some relation to the antitryptic index, though this has not been investigated.

(d) *Globulin-Albumin Ratio.*—According to Loeper and Tonnet,²⁶ and others, cases of malignant disease show an alteration of the globulin-albumin ratio in the serum. Wegievko²⁷ asserts that “the determination of the albumin-globulin quotient must lead to the same result as the flocculation reaction—that is, a serum whose quotient is raised must flocculate easily.” Kennaway²⁸ has, however, shown recently that changes in the globulin-albumin ratio occur only in the serum of advanced cases of cancer. Consequently, this reaction would not appear to depend solely upon this factor.

(e) *Cholesterin Content.*—It seemed possible that the reaction might be due to the cholesterin content of the serum reacting with a colloidal system highly saturated with cholesterin, and that the variations in flocculation might be due to the amount of cholesterin in the different serums. It does not seem to be certain whether there is any constant increase in the amount of cholesterin in the serum in malignant disease. On the one hand, Luden²⁹ and Gordon Reeves³⁰ have found an increase in the amount of cholesterin in the serum of cancer patients, while Falcitore³¹ finds that the amount of blood cholesterin varies according to the site of the tumour, and some, indeed, have found hypcholesterinaemia. In this reaction serums which might be expected to contain large amounts of cholesterin did not give a positive reaction in the absence of malignant disease. Cases of cholelithiasis, which contain a high percentage of cholesterin in the serum, did not react positively. Increase of the amount of cholesterin in the antigen did not change the character of the reaction, but merely increased the sensitiveness of the antigen. It seems that the reaction does not depend solely upon the cholesterin content of the serum.

(f) *Lowering of Surface Tension.*—According to Volta and Benedetti³² colloidal flocculation reactions are due to a lowering of the degree of dispersion of the globulin of the serum; and Holker³³ considered that the interaction of the serum colloids with the colloids of an antigen is in the nature of an adsorption phenomenon between the surfaces of the colloids. E. Bauer³⁴ states that there is a lowering of the surface tension of the blood serum and tissue fluids in carcinomatous diseases. If this is the case, then the formation of aggregates and flocculi through changes in the colloids of the antigen can be readily explained. If, however, it were simply a question of the lowering of the surface tension, one would expect cancer serums to form flocculi with any antigen—for example, the Bordet and Ruelens antigen of the Sigma test. As has been shown, however, this is rarely the case, so that the antigen used in this reaction would appear to have some additional specific properties.

SUMMARY.

1. Over the whole series of 500 cases, comprising malignant and non-malignant conditions, 75 per cent. correct results were obtained.
2. In 239 cases of malignant disease, 170 positive flocculations were obtained (71 per cent. correct).
3. In 261 controls, comprising healthy individuals, cases of non-malignant neoplasms, medical and surgical conditions, 204 gave a negative result (78 per cent. correct).
4. Healthy individuals gave a uniformly negative reaction, except in one instance.
5. Non-malignant tumours usually gave a negative reaction.
6. In the absence of malignant disease flocculations do not in the majority of cases occur with syphilitic conditions, but a control Wassermann reaction or Sigma reaction is advisable.
7. With acute febrile conditions—for example, tuberculosis or sepsis—positive flocculations may occur.

CONCLUSIONS.

1. The preparation of an antigen and arrangement of a simple flocculation reaction has been described. This reaction gives a high percentage of correct results, both in malignant and non-malignant conditions.

2. Excluding certain conditions, the reaction is of value in the diagnosis of malignant disease.

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NASAL, AURAL, AND OTHER FOCAL SEPSIS AS A CAUSE OF NEURASTHENIA AND INSANITY.*

BY

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THE purport of this communication is, first, to invite more widespread attention to sepsis as often the essential cause of functional psychoses, ranging from slight blunting of intellectual activity, through the more definite forms of abnormal taciturnity, inability to concentrate, unsociability, and the varying syndrome of psychic abnormality grouped under the terms "neurasthenia" and "hypochondriasis," to the graver manifestations of unsoundness of mind accompanied by suicidal impulse, delusional insanity, mania, and ultimately dementia praecox; and, secondly, to lay stress on the import of the fact that the sepsis is most frequently what is conveniently termed "focal," and "latent" or "cryptogenic."

Many years ago I held a post of resident clinical assistant at Winsor Green Asylum, where there were about 600 inmates, and to which the acute cases occurring in and around Birmingham were mostly sent. One could not fail to be impressed with some of the acute maniacal cases which were apparently cut short by a blue pill and active treatment of the gastro-intestinal tract. It is now generally accepted that general paralysis of the insane is a legacy of syphilis, and alcohol and toxæmia of gastro-intestinal origin are recognized as causes of functional psychosis of toxic origin. The number of cases of insanity due to apical dental sepsis and cured by extraction of offending teeth is growing rapidly. It is because it is not as yet widely known that focal sepsis of the nasal sinus, tonsil, and ear may cause functional psychosis and insanity that I desire to emphasize the importance of not overlooking such sources of mental trouble, without, however, contending that therein lie the most frequent source of sepsis causing mental aberration. Nor do I touch here on a subject I have elsewhere discussed²—the question of focal sepsis as a cause of mental delinquency and crime—although a fruitful subject if approached with judgement as a matter requiring careful deliberation. On defective delinquency and functional psychosis the splendid work carried on at the State of New Jersey's mental hospital, as described by Dr. Henry Cotton,³ is worthy of most careful attention.

In 1907 in the Long Fox Lecture¹ I stated that—

"All patients suffering from purulent sinusitis are liable to suffer from general toxæmic symptoms, toxic products reaching the blood either from direct absorption from the implicated sinuses or from

the gastro-intestinal tract, but lymphatic absorption of toxic matters, in the presence of channels of communication with the intracranial venous sinuses, must be held to account for the profound mental depression and difficulty in thinking clearly, almost amounting to slow cerebration, that is very frequently present in fronto-ethmoidal or spheno-ethmoidal sinus suppuration, and sometimes a less marked degree, even in simple maxillary sinus disease. Many patients have come with drawn, sad expression and sallow complexion, expressing their weariness of life and a profound melancholia which was quite foreign to their natural state of mind—symptoms which have completely disappeared with the removal of their sinus disease; sometimes it has been difficult to recognize in the round-faced cheerful individual one who shortly before treatment was a haggard melancholic."

Up to sixteen years ago I had never met with a case where definite mental aberration preceded and was relieved by accessory sinus treatment, though many of my patients were the subjects of a psychosis and several had confessed to strong suicidal impulses. Shortly after this I encountered examples of definite insanity. The most remarkable in my experience occurred in 1911. It will be more useful to relate this one case at some length than cite many cases, some of which I have already published.¹⁰

A healthy-looking, well nourished man, aged 29, keen and good at outdoor games, shooting, and so forth, had a severe attack of influenza in 1901. In 1903 he had his first definite appendicitis. Appendicectomy was performed in 1907. This was followed by insomnia, for which he went on a voyage to Madeira.

Recovery seemed complete, and till early 1911 he was very bright, full of fun, and keen on politics and his work. (? Acquired relative immunization.) In February, 1911, he had influenza with bad headaches and general malaise, but not badly, so did not stay in bed, and went on till insomnia compelled him to give up work and go away for five weeks. He returned thin, miserable, and depressed. He tried to work for the next two months, his doctor advising this "as it would take his mind off himself"; but he found it impossible to continue. He was sent on a voyage, but "seemed in a mental mist all the time" and terribly depressed. (Later, he told me, he took a pistol with him intending to shoot himself.)

When I saw him in August, 1911, he looked ruddy and well nourished, but had been obliged to give up work, though he still played fairly good golf. But he said his memory was gone, he could not think, and his "mind was a blank." He had post-nasal discharge, which he had been told was of no importance. I found fetid pus in the one antrum explored. He travelled to Bristol, wandering up and down the train corridor the greater part of the night, and could not sit still. He declared he was "devil driven," and that "it was a sin to have an operation, because he was dead." Mr. Stock gave an anaesthetic on September 23rd, 1911, and I opened both maxillary antra, which contained fetid pus, and from the frontal sinuses pus was washed out. Lavage of frontal sinuses and antra was continued daily, but his delusions became more insistent. He refused all food because "he was dead." The matron of the home said she feared to keep him because he was insane, and ought to be under certificates; but, instead of certifying, with his parents' consent I operated again on October 29th, assisted by Mr. A. J. Wright, performing my radical osteoplastic flap-frontal sinus operation. The frontal sinuses were unusually large and the mucosa much diseased. Cultures from the antra and frontal sinuses yielded pure *Staphylococcus aureus* (J. Walker Hall).

For several weeks he refused all food and had to be forcibly fed by male attendants; he tore off his dressing when he got the chance.

He went home on December 3rd, with the operation wounds healed, mentally much improved, but still subject to delusions of having "committed unpardonable sins"; even then at times he refused food and declared he was dead. His delusions continued for some months, but they became progressively less dominant, and less than a year later he was writing sensible letters full of interest in life. Very soon after that he was back at his work; since then he has remained in excellent health and has occupied a responsible position for a period of twelve years.

I would emphasize the following points in this case:

(a) The long history of sepsis following influenza in 1901, with gastro-intestinal infection and appendicitis probably of nasal origin—a by no means infrequent occurrence.

(b) After a period during which the infection was inhibited by auto-immunization a second attack of influenza caused a relapse and the nasal sinus infection caused profound mental disturbance—melancholia with resisted suicidal impulses. Despite a prolonged holiday and a voyage, he could no longer regain the power of work, and he then developed delusions.

(c) He did not lose his delusions for several months after disinfection and detoxication, but after his general health had been restored he regained all his old mental activity and geniality and has continued to occupy a position of responsibility without intermission.

(d) The patient was undoubtedly insane, and apart from the symptoms related, the letters in my possession that he

* This paper and the three papers that follow it were read before the Bath and Bristol Branch of the British Medical Association at the University of Bristol, May 27th, 1925.