fectants emphasises that certain precautions are necessary to prevent contamination of these products. Containers must be carefully cleaned before they are refilled, any stock solutions (i.e., any dilute solutions that are not for immediate use) should contain either 7% v/v ethanol or 4% of isopropanol, and contact between disinfectant and corks or cork cap-liners must be avoided.

The hospital decided to issue chlorhexidine-containing disinfectants only as heat-sterilised solutions in small-capacity containers. This step was taken in view of the likelihood of continued introduction of the organism by way of the water supply, and the ability of the organism to multiply in the disinfectant. It should be a sufficient precaution against contamination provided serious abuses are avoided, such as the prolonged retention of bottles of dilute disinfectant for occasional use and the refilling or " topping up " of such bottles in the wards or departments. In 12 months since this precaution was taken there has been no further incident of infection with this organism.

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SKIN-REACTIVE SOLUBLE ANTIGEN FROM INTESTINAL CANCER-CELL-MEMBRANES AND RELATIONSHIP TO CARCINOEMBRYONIC ANTIGENS

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Summary Delayed hypersensitivity reactions in seventeen of nineteen patients with carcinoma of the colon and rectum were elicited by soluble fraction obtained from the membranes of autologous tumour cells. Negative reactions were obtained with comparable fractions obtained from normal cells. Skin-reactive antigen was also detected in the digestive-tract cells of first-trimester and second-trimester fetuses. Carcinoembryonic antigen (C.E.A.) was detected in many of the fractions producing positive skin reactions. The skin-reactive tumour antigen appeared to be closely related, and possibly identical, to the C.E.A. but, although found in comparable fractions, it may well be distinct from the C.E.A.

Introduction

Preliminary studies have been made of certain fractions of partially separated soluble-membrane antigens from animal and human cancer cells. Such antigens appear to afford protection to tumour-cell challenge in animals or to elicit a specific cutaneous hypersensitivity reaction in man, whereas similar fractions of soluble-membrane antigens of an equal protein content from normal tissue do not elicit such responses.[1-3]

In the present study, skin tests were performed on patients with carcinoma of the rectum and colon in an attempt to detect cellular-immunity (delayed-hypersensitivity) reactions to tumour antigens. Delayed-hypersensitivity reactions to antigens on human tumour cells have been reported by several investigators.[4-9] All of these studies were performed with cell extracts containing surface membranes or other particulate material. The tumour specificity of the observed skin reactions has not been conclusively demonstrated; some reactions have been obtained with control extracts as well as with tumour-cell extracts. In this study soluble extracts were prepared from whole cells or from membrane extracts, and this allowed more definitive demonstration of tumour specificity.

It was also possible to compare the soluble membrane antigens with the carcinoembryonic antigen (C.E.A.) of Gold. Gold et al. have shown that certain C.E.A.s of the human digestive system are found only in adenocarcinomas arising from the entodermally derived digestive-system epithelium and in embryonic and fetal gut, pancreas, and liver tissue during the first two trimesters of pregnancy.[10-12] This C.E.A. appears to be a glycoprotein closely associated with the cell surface-membrane.[13] 70% of patients with non-metastatic cancers of the digestive system have anti-C.E.A. antibodies in their sera as measured by agglutination of erythrocytes chemically coupled to C.E.A. Antibody titres did not correlate, however, with the clinical status of the patient.[11] In addition to these studies by Gold, he has recently described the presence of the C.E.A. antigens in the blood-stream; in blood tests, by blindscreening of 200 patients with various diseases, the antigen was easily detectable in the 35 patients with colon and rectal cancer.[14]

Materials and Methods

Preparation of Soluble-membrane Antigens

Fetal digestive tracts were obtained from first, second, and third trimester therapeutic abortions, from mothers with no history of any drugs which might induce chromosomal aberrations in the fetal tissue. The fetuses were obtained either by aspiration or by surgical removal from the intact uterus after hysterotomy. In each patient at the time of operation rectal and colonic cancer and normal tissue were obtained to be skin-tested. The fetal gut, adult carcinoma, and normal tissues were washed for ten minutes at 37°C five times in physiological saline solution. The cells were teased into suspension and counted. Membrane extractions were performed by the procedure des-

cribed by Davies [15] or a modification of this method.[8] Sterility was monitored for each step by thioglycollate and blood-agar plate testing. The fetal membranes were incubated overnight in the refrigerator with pooled normal human sera selected for alloantibody content, centrifuged at 100,000 *g*, washed, and centrifuged again. Solubilisation procedures using low frequency sonication and ' Sephadex ' gel filtration procedures have been described.[1,16,17] The membrane pellets were resuspended in saline solution, sonicated at low frequency for three minutes, and centrifuged at 100,000 *g*, and the pellet was resuspended and sonicated again for three minutes and centrifuged. The pooled soluble antigens were separated on sephadex G-200, with spectrophotometer monitoring of the 2 ml. eluates at 280 mμ for protein content and also at 220 mμ in order to measure non-aromatic aminoacids and other material. The void volume was approximately 25 ml. The eluates were pooled into four separate fractions of 20 ml. each. Most of the protein was in fraction III, but in some separations there was also considerable protein in fraction II. The 220 mμ/280 mμ ratio of fraction III was greater for the fetal fractions than for the adult fractions. Control curves with known proteins were plotted for 2 ml. eluates and an approximate molecular weight, according to the method of Whitaker,[16,18] was calculated. Although much further separation and purification is required before a definite calculation can be made, in general the peak of fraction III was approximately 175,000 molecular weight. For certain experiments a second separation of fraction III yielded another peak the center of which was approximately 88,000 molecular weight.

Protein determinations were made by the method of Lowry, and concentrations of the material for a similar protein content between specimens were adjusted using dilution or ' Diaflo ' ultrafiltration.

Delayed-hypersensitivity Testing

The delayed-hypersensitivity testing [7] in patients with rectal and colonic carcinoma involved intradermal injection of 0·1 ml. of autologous tumour and control cell-free soluble-membrane antigens and mumps antigen into the patient's back, and observations of both erythema and induration were recorded at 2, 12, 24, 48, and 72 hours in order to compare the initial and delayed reactions. A delayed-hypersensitivity skin reaction of 5 mm. or greater was considered positive. Biopsies of some positive skin reactions were obtained; one of the authors and a pathologist reviewed all skin-biopsy findings independently.

Carcinoembryonic antigen (C.E.A.) tests were performed by one of us (P. G.) by described methods.[14] All samples were processed on the basis of a double-blind design.

Assay for HL-Alloantigenic Activity

Membrane and soluble preparations were assayed for HL-A alloantigenic activity by their ability to inhibit the cytotoxic activity of a broadly reactive antiserum designated lochum. This antiserum and the method employed has been described in detail by Mann et al.[19] The human lymphoid tissue-culture cells, IM-I, were labelled with ^{51}Cr and used as the target cells. 10μl. of the antiserum dilution capable of producing 70% release of ^{51}Cr was added to 10 μl. of antigen preparation and the mixture was incubated at room temperature for 15 minutes. 10 μl. of ^{51}Cr-labelled cells (10^4) and 10 μl. of undiluted rabbit serum as a source of complement were added and the incubation was continued for 45 minutes at 37°C. The amount of ^{51}Cr released from the cells was then counted. A unit of alloantigenic activity was defined as the amount of material needed to cause 50% inhibition of the ^{51}Cr release.

Results

Eighteen patients were tested with pooled and concentrated soluble fractions of their autologous tumour and control membrane extracts (table I). Sixteen had positive delayed reactions to soluble fraction III of the tumour. In four patients there was also a positive reaction to tumour-soluble fraction II, but these reactions faded away by 48 hours. Two patients gave equivocal or negative reactions; but they also gave negative reactions to mumps antigen and many have been anergic. No patient gave persistent positve reactions to the control fractions; one patient had some reactivity to fractions II and III at 24 hours, but these reactions became negative by 48 hours.

TABLE I—DELAYED-HYPERSENSITIVITY REACTIONS TO SOLUBLE FRACTIONS OF TUMOUR AND CONTROL CELL MEMBRANES

Patient	Soluble sephadex-separated fractions								Mumps
	Cancer				Normal				
	I	II	III	IV	I	II	III	IV	
A	−	−	+	−	−	−	−	−	+
B	−	±	+	−	−	−	−	−	+
C	−	−	+	−	−	−	−	−	+
D	−	±	+	−	−	−	−	−	N.T.
E	−	±	+	−	−	−	−	−	N.T.
F	−	−	+	−	−	−	−	−	+
G	N.T.	−	+	N.T.	N.T.	−	−	N.T.	+
H	N.T.	−	+	N.T.	N.T.	−	−	N.T.	+
I	N.T.	−	±	N.T.	N.T.	±	±	N.T.	±
J	N.T.	−	+	N.T.	N.T.	−	−	N.T.	N.T.
K	N.T.	−	+	−	−	−	−	−	N.T.
L	N.T.	−	+	N.T.	N.T.	−	−	N.T.	N.T.
M	N.T.	±	±	N.T.	N.T.	−	−	N.T.	±
N	N.T.	−	+	N.T.	N.T.	−	−	N.T.	+
O	N.T.	−	+	N.T.	N.T.	−	−	N.T.	+
P	N.T.	−	+	N.T.	N.T.	−	−	N.T.	+
Q	N.T.	−	+	N.T.	N.T.	−	−	N.T.	+
R	N.T.	−	+	N.T.	N.T.	−	−	N.T.	+

N.T.: not tested. +: > 5 mm. induration at 24 and 48 hours. ±: > 5 mm. induration at 24 hours. −: < 5 mm. induration.

Biopsies of the positive skin reactions showed histological reactions compatible with delayed hypersensitivity reactions. There were marked perivascular and histocyte infiltrates in the biopsy specimens of reactions to tumour-soluble fraction III.

Because of the observations of Gold et al. [10–12] that C.E.A. was present in fetal gut, further skin tests were then performed on two patients (A and B) with pooled membranes and soluble fractions of first and second trimester fetal endothelium. As indicated in table II, the protein concentration of each inoculation was 126-284 μg. per 0·1 ml. The delayed reactions were measured at 24 and 48 hours after inoculation. An induration of greater than 5 mm. diameter was considered positive; and no attempt was made to measure the reaction three-dimensionally, since induration was sometimes in the form of a papule and at other times in the form of a plaque. Erythema was observed; the intensity at 12 hours was a pronounced red, by 24 hours was pale pink, and by 48 hours was rather difficult to assess. Of the two patients, patient A had positive reactions to tumour membranes and soluble fraction III from the tumour membranes. He also had a reaction to the fetal gut membranes and soluble fraction III from fetal gut membranes. The reactions to the fetal materials, however, were maximal at 24 hours and were considerably smaller by 48 hours. Negative reactions were seen to the control membranes and soluble fractions. Similar results were obtained in patient B. In this patient the reactions to the fetal membranes and soluble fraction III of fetal origin re-

mained positive at 48 hours, with histological confirmation for a reaction consistent with those seen for delayed hypersensitivity with tumour fraction III.

In view of these results with the pooled first and second trimester fetal materials, it was of interest to test the fetal membranes from a third-trimester fetus. Since only one third-trimester fetus was obtained, there was insufficient membrane material to prepare soluble fractions. Tests were only performed with the membranes in patient C. The fetal membranes gave a negative response, whereas the membranes of the autochthonous rectal carcinoma elicited a positive reaction.

Because the distribution of the antigen detected by the delayed-hypersensitivity skin-testing was quite similar to that observed for the carcinoembryonic antigen, it seemed possible that C.E.A. was producing the positive skin reactions. Membrane and soluble fractions were therefore tested by one of us (P.G.) for the presence of C.E.A., by the radioimmunoassay.[14] As shown in table II, carcinoembryonic antigen was detected in all of the rectal carcinoma materials, including fraction II which did not produce a positive skin reaction. C.E.A. was not detected in the normal rectal materials nor in the fetal soluble fractions. These fetal preparations were from abortions by aspiration, and the gut tissue was of poor quality. The quantity of C.E.A. present might have been below the level of detection.

TABLE II—INITIAL AND DELAYED HYPERSENSITIVITY REACTIONS IN PATIENTS TESTED WITH FETAL, NORMAL RECTAL, AND RECTAL CANCER CELL MEMBRANES, AND SOLUBLE MEMBRANE FRACTIONS

Membrane and soluble membrane antigens	Initial and delayed cutaneous hypersensitivity reactions						Amount of protein (μg./0·1 ml.)
	12 hr.		24 hr.		48 hr.		
	E†	I‡	E†	I‡	E†	I‡	
PATIENT A							
1st and 2nd trimester fetal endothelial membranes:	10	10	10	5	5	2	186
Fetal fraction I	8	8	4	0	0	0	284
Fetal fraction II	8	5	10	0	0	0	188
Fetal fraction III	10	10	13	13	4	4	270
Fetal fraction IV	10	10	4	0	0	0	226
Normal rectal membranes:	10	8	8	3	4	0	184
Normal fraction I	11	7	6	3	3	0	160
Normal fraction II	10	10	5	4	2	1	148
Normal fraction III	10	10	6	0	4	0	126
Normal fraction IV	8	7	3	0	0	0	136
Rectal cancer membranes:	12	10	10	10	10	8	204
Rectal cancer fraction I	11	6	8	0	4	0	200
Rectal cancer fraction II	10	3	10	0	6	0	193
Rectal cancer fraction III	11	11	10	8	6	5	179
Rectal cancer fraction IV	10	7	4	0	2	0	168
PATIENT B*							
1st and 2nd trimester fetal endothelial membranes:	..	..	8	6	5	5	186
Fetal fraction II	..	..	7	2	3	0	188
Fetal fraction III	..	..	10	10	7	5	270
Normal rectal membranes:	..	..	11	3	7	0	296
Normal fraction II	..	..	9	3	5	2	254
Normal fraction III	..	..	10	0	5	0	270
Rectal cancer membranes:	..	..	9	7	4	4	230
Rectal cancer fraction II	..	..	7	3	0	0	202
Rectal cancer fraction III	..	..	6	6	6	6	184
PATIENT C							
Rectal cancer membranes:	12	12	12	10	10	10	230
3rd-trimester fetal membranes:	10	10	8	3	3	0	192

* Hour 12 not recorded. † E: erythema (mm.). ‡I: induration (mm.).

TABLE III—STUDY FOR THE PRESENCE OF C.E.A. ANTIGENS IN FETAL, NORMAL, AND CANCER CELL MEMBRANES AND SOLUBLE MEMBRANE FRACTIONS

Material*	Protein concentration (μg./0·1 ml.)	Delayed skin reaction	C.E.A.
Rectal cancer membranes 1:10 ..	23	N.D.†	+
Rectal cancer membranes	230	+	+
Rectal cancer soluble fraction II ..	202	−	+
Rectal cancer soluble fraction III ..	184	+	+
Normal rectal membranes 1:10 ..	29	N.D.	−
Normal rectal membranes ..	296	−	−
Normal rectal soluble fraction II ..	254	−	−
Normal rectal soluble fraction III	270	−	−
Fetal membranes	186	+	N.D.
Fetal soluble fraction II	188	−	−
Fetal soluble fraction III	270	+	−

* Patient B, table I. † Not done.

An additional study was performed with another patient with carcinoma of the colon. Membranes were prepared from the intestinal and from pooled œsophageal and gastric tissues from intact fetuses obtained by hysterotomy. The patient was skin-tested with the autologous tumour and control materials, the fetal materials, and purified C.E.A. These preparations and some other control preparations (from leiomyosarcoma of stomach and normal gastric mucosa) were also tested for the presence of C.E.A.and for the presence of HL-A histocompatibility antigens. Table IV summarises the results of these tests. The patient gave positive skin reactions to the tumour membranes and soluble fraction III, bringing the total to 17 out of 19 patients showing this specific reaction to soluble fraction III. Positive reactions were also obtained with the first and second trimester fetal membranes. The normal rectal membranes and soluble fractions and the third-trimester

TABLE IV—FURTHER TESTS OF THE ACTIVITY OF FETAL, ADULT NORMAL, AND CANCER CELL MEMBRANES AND SOLUBLE ANTIGENS: COMPARISON OF DELAYED-HYPERSENSITIVITY, CYTOTOXIC INHIBITION AND C.E.A. ACTIVITIES

Material tested	Protein conc. (μg./0·1 ml.)	Delayed hypersensitivity*	Presence of C.E.A.	Cytotoxic inhibition (units/mg. protein)†
Colon cancer membranes ..	300	+	+	++(166)
Cancer fraction II	140	−	−	0(0)
Cancer fraction III	284	+	+	+++(147)
Cancer fraction IIIa	70	+	N.D.	+(32)
Cancer fraction IIIb	64	−	N.D.	+(20)
Cancer fraction $IIIb_2$	59	−	N.D.	+(19)
Normal rectal membranes ..	256	−	−	+(10)
Normal fraction II	100	−	−	+(10)
Normal fraction III	130	−	−	0(0)
1st-trimester fetal intestine membranes	310	+	+	++++(332)
1st-trimester fetal intestine membranes 1:10	31	+	N.D.	N.D.
2nd-trimester fetal intestine membranes	249	+	−	+(40)
3rd-trimester fetal intestine membranes	225	−	−	0(0)
1st-trimester fetal stomach and œsophagus membranes ..	34	+	+	+++(294)
2nd-trimester fetal stomach and œsophagus membranes ..	52	+	−	++(150)
Leiomyosarcoma membranes ..	465	N.D.	−	++++(344)
Normal gastric mucosa membranes	160	N.D.	−	++++(375)
C.E.A. antigen	250	−	..	+(28)

* Induration > 5 mm. 24–48 hours. † 0: negative.
+: 15–25% inhibition. ++: 26–50% inhibition.
+++: 51–75% inhibition. ++++: 76–100% inhibition.

fetal membranes gave negative reactions. C.E.A. was detected only in materials producing positive skin reactions. However, C.E.A. was not detected in the second-trimester fetal membranes—perhaps a reflection of the lower C.E.A. content after the first trimester. It should be noted that a negative skin reaction was obtained with purified C.E.A.

The soluble cancer fraction III, obtained by sephadex G-200 chromatography, contained skin-reactive antigen, HL-A antigen, and C.E.A. antigen. We were interested in further separation and possible purification of the skin-reactive antigen. Fraction III was therefore recycled on sephadex G-200 (see Methods). One protein peak (IIIa) was in the same molecular-weight range as that of the original fraction III, and another peak (IIIb) eluted in the range of half that molecular weight. A portion of each pool was concentrated and saved for skin-testing. The rest of the second pool (IIIb) was put on a sephadex G-100 column in order to ascertain the homogeneity of this material. A single peak ($IIIb_2$) eluted. These materials were adjusted for protein concentration, and 0·1 ml. of each was inoculated into the patient. Only the concentrated eluate of the higher-molecular-weight pool (IIIa) elicited a positive delayed hypersensitivity reaction (table IV). HL-A antigenic activity was found in both the high and low molecular-weight pools.

Discussion

These results indicate that patients with carcinomas of the colon and rectum have delayed-type or cellular immunity to antigens present on the tumour cell membranes. These antigens appeared to be tumour-specific since comparable reactions were not obtained with autologous control antigens. These reactions were noted after operative removal of the tumours. Further tests will be needed, at other stages of disease, to determine whether the cellular immune reactivity is related to clinical state. Previous studies with patients with Burkitt's lymphoma and with melanoma have suggested such a relationship.[6,7]

In the present study the skin-reactive tumour antigen was obtained in soluble form by low-frequency sonication. After chromatography on sephadex G-200, activity was consistently found in fraction III, which had an estimated molecular-weight range of about 175,000. In some cases a lesser degree of reactivity was also noted in fraction II, but this could be explained by incomplete separation.

The studies indicated a possible relationship of the skin-reactive antigen with the carcinoembryonic antigen. As observed with C.E.A.,[10–12] skin-reactive antigen was detected in first and second trimester fetal endothelial separations. The previous demonstration of the close association of C.E.A. with the cell surface-membrane [13] was confirmed by the detection of C.E.A., as well as skin-reactive antigen, in the cell-membrane extracts. The C.E.A. and the skin-reactive antigen also separated together on sephadex G-200, both being present in fraction III. This fraction was shown to contain more than one type of antigen, since considerable HL-A antigenic activity was also present. It remains to be determined whether the skin-reactive tumour antigen and C.E.A. are the same, or two distinct antigens, with similar physicochemical properties and similar biological distributions. The patient in table IV had a negative skin reaction to purified C.E.A., yet had a positive reaction to autologous tumour materials. Possibly the harsh extraction and purification procedures used to isolate the C.E.A. caused some change in the C.E.A. which, although not affecting its serological reactivity, could have made it less able to elicit a skin reaction. Other purification procedures will be needed to define the relationship between the skin-reactive antigen and C.E.A.

We are impressed with the fact that it may be necessary to have a complex protein in order to elicit delayed-hypersensitivity reactions. It is well known, in studies of drugs, that simple chemicals cannot induce immune responses, and that it is necessary to have a hapten-protein conjugate in order to induce immunogenicity.[20] Another possibility might be that, with further purification, a carrier is either eliminated or altered. Sela [21] has stated " that an antigen is much more than just an antigenic determinant attached to an inert carrier, and that the carrier moiety of the molecule, while not contributing to specificity, plays an important role in defining the biosynthesis and structure of the antibodies formed." Most of the studies of solid tumours show that the investigators have been unable to induce tumour rejection using isolated small membrane fragments, whereas the larger fragments appeared to elicit specific resistance to tumour challenge in animals. We have found such methods as nitrogen decompression, prolonged sonication, or enzyme digestion, or harsh homogenisation procedures, to be useless. It is only by the gentle extraction of membranes, and by careful sonication, enzyme digestion, or salt extraction procedures, that one can obtain high yields of soluble antigens.[22] Both the higher molecular-weight component (fraction IIIa) and the lower-molecular-weight component (IIIb) were positive in the cytotoxic inhibition test (table IV), perhaps indicating that an in-vitro antigenic determination can be made even though the immunogenic capacity is lacking. Another possibility is that the lower-molecular-weight component is a contaminant antigen, unrelated to the larger component. Nevertheless, these results indicate that it will be most difficult to further separate and identify such tumour-specific antigens, in that the antigens are complex and, perhaps, associated with carriers necessary for their in-vivo behaviour. It will be equally difficult to determine whether or not such tumour-specific antigens are altered or enhanced histocompatibility antigens.

It is apparent that the fetal-cell-membrane antigens are in some manner related to the tumour-specific transplantation antigens being studied in adult tumour cells, and are also closely related in antigenic structure to those antigens (C.E.A.) detectable in the sera of patients with intestinal cancer. We are continuing studies of the genetic and immunological relationships of these antigens. It must be emphasised that the C.E.A. and tumour-specific transplantation antigens may be two separate components.

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SUPRAPUBIC ASPIRATION OF URINE IN DIAGNOSIS OF URINARY-TRACT INFECTIONS DURING PREGNANCY

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Summary The withdrawal of urine by suprapubic aspiration has proved to be a suitable method where it is important to obtain a specimen free from contamination. From a study of 429 such specimens obtained from pregnant or recently delivered women it was found that 65% of the sterile specimens came from patients with symptoms suggestive of urinary-tract infection (U.T.I.). Of all the cases with symptoms only 41% actually excreted bacteria in the urine. Of the symptoms considered only pyrexia was commoner in infected cases. It is concluded that the traditionally accepted symptoms and signs are of little help in diagnosing U.T.I. in pregnancy and that it is necessary to demonstrate the presence of bacteria in an uncontaminated specimen.

Introduction

IN this report we discuss the correlation of the signs and symptoms found in a series of 306 pregnant or recently delivered women with the presence or absence of bacteria in the urine. The specimens were obtained by suprapubic aspiration of the bladder.

Cultures of midstream specimens of urine (M.S.U.) are generally used in the diagnosis of urinary-tract infection (U.T.I.). However, the use of samples obtained by suprapubic aspiration of the bladder has been described by several workers,[1-5] and its value in pregnancy has been investigated by McFayden and Eyken.[6] In a small pilot trial we found that 30% of the patients would have been regarded as having U.T.I. but for the results obtained by suprapubic aspiration (table I). In only 4 instances where the urine was collected by suprapubic aspiration were bacteria found when the M.S.U. was sterile; in all 4 instances the organism was *Staphylococcus epidermidis* and numbered less than 10,000 per ml. In the positive M.S.U.s at least 100,000 organisms per ml. were present. As a consequence of this result we decided to use specimens obtained by suprapubic aspiration in the investigation of pregnant women.

TABLE I—COMPARISON OF SPECIMENS OBTAINED BY SUPRAPUBIC ASPIRATION AND M.S.U. IN 90 PATIENTS

Suprapubic aspiration	M.S.U.	No.
+	+	35
−	−	24
−	+	27
+	−	4

Patients and Methods

429 examinations were done on 306 patients. Of the 429 specimens, 363 came from antenatal patients and 66 from patients in the first week of puerperium. There were three indications for examination:

(1) Symptoms and signs usually regarded as suggestive of U.T.I.—viz., frequency of micturition, dysuria, loin pain, pyrexia, hæmaturia, stress incontinence, and lower abdominal pain.
(2) A history of previous U.T.I.
(3) As part of the investigation of anæmia or hypertension.

Collection of Urine

The patients were encouraged to drink and to refrain from micturating. When the bladder was palpable the skin was cleaned with surgical spirit and the urine withdrawn using a 21 gauge disposable needle on a 20 ml. syringe. Local anæsthetic was not used.

Cell-count

If the urine seemed clear to the naked eye it was centrifuged and the deposit from 10 ml. was resuspended in 1 ml. of saline solution before counting in a modified Fuchs-Rosenthal chamber; if the specimen was turbid, the cells were counted without centrifugation.

Culture

1 ml. urine was placed in nutrient broth; 0·1 ml. urine was streaked on a horse-blood nutrient agar plate, and successive dilutions (10^{-3} to 10^{-11} ml.) were similarly plated. Incubation was at 37°C for eighteen hours.

Results

The results are set out in tables II and III.

Relation of Symptoms to Presence of Bacteria

Of 297 patients with symptoms suggestive of U.T.I., only 122 (41%) actually excreted bacteria in the urine.

Of 160 patients with bacteriuria, 122 (76%) had symptoms usually regarded as suggestive of U.T.I.;

TABLE II—FREQUENCY OF SYMPTOMS IN ASSOCIATION WITH INFECTED AND NON-INFECTED URINE

—	Infected urine	Non-infected urine	Total
Symptomatic	122	175	297
Symptom-free ..	38	94	132
Total	160	269	429