cm.) from the external cervical os, under direct vision; and under maximum pressure the liquid was sprayed on to the squamo-columnar junction. The liquid was then aspirated from the posterior fornix, and this procedure was repeated three times.

The washing (5–6 ml.) was filtered through coarse gauze to remove debris and mucus, and about 0.1 ml. of the filtrate was spread on a slide, air-dried, fixed in methanol, and stained with Papanicolaou stain. The remainder of the filtrate was diluted with 0.85% saline to give a cell count suitable for the counter.

The size-distribution patterns were determined by means of the Coulter counter model B with automatic plotter. The performance of this equipment was regularly checked with a double pulse generator and by repeated use of test particles from the same batch. The Coulter counter and plotter have been described in detail (Kubitschek, 1960; Brecher, Jakobek, Schneiderman, Williams, and Schmidt, 1962).

The cervical washings were obtained from patients attending gynaecological and post-natal clinics and from patients admitted to the Royal Beatson Memorial Hospital for radiation treatment. Smears and biopsy specimens were examined by the pathological departments concerned, and one of (S. I.) examined the slides from the washings.

Results

The results of the non-cancer cases are summarized in Table I, and those of the cancer cases in Table II. Some of the latter were examined on two occasions with an interval of one week between washings, and one case on three occasions.

The results from the cancer patients are obviously disappointing. For example, of 30 cases of cancer of the cervix 19 were Coulter-positive and 11 Coulter-negative at the first examination. Assuming an even chance for a Coulter-positive or a Coulter-negative response the probability of the occurrence of such frequency is 0.10—that is, not significantly different from the assumption. The results from the second examination of cancer of the cervix are also not significant ($P=0.073$).

Our results differ considerably from those of Ladinsky *et al.* (1964) and we have therefore tried to find a reason for this. Re-examination of the slides from the cervical washings made it apparent that all the Coulter-positive washings from cancer and non-cancer cases contained more leucocytes than epithelial cells of any type. On the other hand, the Coulter-negative washings either contained a large number of erythrocytes—far in excess of any epithelial cell type—or showed only predominance of epithelial cells. This suggests that a Coulter-positive

TABLE I.—Results in Non-cancer Cases. Coulter-positive or Coulter-negative Indicates the Presence or Absence of a Second Cell Population, as Defined by Ladinsky *et al.* (1964)

	Coulter		
	Negative	Positive	Doubtful
Negative smear	91	2†	5‡
Clinically healthy (no smear available)	28		
Positive smear	1*		

* Biopsy negative.

† On repeat 2–3 months later, Coulter-negative.

‡ Second population with modes 480–580 μ^3 . On repeat 2–3 months later they were all Coulter-negative.

TABLE II.—Results in Cancer Cases

Examination:	1st		2nd		3rd	
Coulter:	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Carcinoma of cervix	19	11	9	3	1	0
Adenocarcinoma of uterus	2	6	1	4		
Cancer of vagina	2	6				
	23	23	10	7	1	0

pattern is not by itself an indication of malignancy but of the presence of leucocytes in sufficient number in the washing.

This is corroborated by the finding that leucocytes isolated from human blood gave size distributions with modal values in the range 620–860 μ^3 (depending upon the osmolarity of the solution), which are well within the modal values for the Coulter-positive washings. The variance of the distributions of the latter was greater than that of isolated leucocytes. A sample of human pus from an abscess gave a modal value corresponding to 740 μ^3 .

Conclusions

We conclude from these results that a Coulter-positive pattern depends more upon the number of leucocytes present in the cervical washing than on the presence of normal or malignant cells, and that, therefore, determination of cell size-distribution by this method in its present form is not a reliable screening method for cancer of the cervix or for any other form of cancer of the female genital tract.

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Preliminary Communications

Serum Alkaline Phosphatase in Pregnancy: An Immunological Study

Brit. med. J., 1966, **1**, 1210–1212

Evidence is accumulating that in the later stages of pregnancy placental alkaline phosphatase is present in the maternal circulation. Maternal serum alkaline phosphatase resembles placental alkaline phosphatase in its heat stability (McMaster *et al.*, 1964), substrate specificity (Sadovsky and Zuckerman, 1965), migration on starch gel (Boyer, 1961), and resistance to chemical inactivation (Kitchener *et al.*, 1965). Serum alkaline phosphatase in the foetus shows none of these characteristics.

In order to provide specific evidence for the identity between placental alkaline phosphatase and the enzyme in the maternal

circulation an anti-human placental alkaline phosphatase antibody was prepared in the rabbit. This paper describes our experiences with this preparation.

MATERIALS AND METHODS

Placental alkaline phosphatase was purified by the method of Ahmed and King (1960). The powder obtained after the first acetone precipitation had a specific activity of 40 King-Armstrong (1934) units per mg. of protein, and this was used without further purification.

The powder was dissolved in Ringer-lactate, containing 1:10,000 Merthiolate (Lilly) so as to give a concentration of 2–3 mg. of protein per ml. One part of this solution was well mixed with two parts of Freund's adjuvant (Kabat and Mayer, 1961) so as to form a water-in-oil emulsion. This was injected

subcutaneously into rabbits in three weekly doses of 1 ml. each. Blood was collected three to eight days after the last injection and the gamma-globulin from the pooled sera was precipitated by the sodium sulphate fractionation method of Kekwick (1940). The material obtained by this procedure, which appeared as a single band in the gamma-globulin region after paper electrophoresis, was dissolved in Ringer-lactate and stored deep-frozen until required.

The antibody thus obtained had no effect when incubated with alkaline phosphatase from *Escherichia coli* prepared by the method of Garen and Levinthal (1960) or with alkaline phosphatase from unpurified human bone extract. It inactivated crude human placental extract, semi-purified human placental alkaline phosphatase (Ahmed and King, 1960), and human placental alkaline phosphatase which had been infused into human subjects (Clubb *et al.*, 1965) to approximately 5% of their original activities (Birkett, 1964). Human intestinal alkaline phosphatase purified by the method of Grossberg *et al.* (1961) was inactivated to about 50% of its original activity after incubation with the antiplacental antibody.

The following sera were investigated by means of the antiplacental preparation: sera from 10 apparently healthy blood donors; cord sera from 10 normal newborn infants; and sera from 10 pregnant women, taken within 24 hours of confinement: the latter were divided into two fractions, one of which was heated at 56° C. for 30 minutes (McMaster *et al.*, 1964).

Of the gamma-globulin solution 0.1 ml. was added to 2 ml. of each serum; 0.1 ml. of 0.9% saline was added to the control sera. The mixtures were incubated for 30 minutes at 37° C., and were then left for 96 hours at 4° C. At the end of this time they were centrifuged at 4,000 r.p.m. at room temperature for 20 minutes and the supernatant was assayed for alkaline phosphatase activity by the automated method of Marsh *et al.* (1959) as modified by Kitchener *et al.* (1965).

RESULTS

Table I shows the action of the anti-human placental alkaline phosphatase antibody on the four groups of sera investigated. It is seen that sera of normal subjects and newborn infants are not appreciably affected by this preparation. Alkaline phosphatase activity in the sera of pregnant women diminished by

TABLE I.—Alkaline Phosphatase Activities (King-Armstrong Units per 100 ml.) Remaining After Incubation of Four Groups of Sera with Anti-human Placental Alkaline Phosphatase Antibody

Source of Sera	No. Investigated	Control Sera Mean Activities ± 1 S.D.	Antibody-treated Sera Mean Activities ± 1 S.D.
Normal subjects ..	10	7.5 ± 1.7	7.0 ± 1.5
Newborn infants ..	10	14.4 ± 3.8	13.7 ± 3.6
Pregnant women ..	10	18.1 ± 10.8*	11.0 ± 9.6
Pregnant women (sera pre-heated) ..	10	8.3 ± 8.6*	1.2 ± 0.8

* The high-standard deviations are due to one high value (see Table II). There is evidence (Kitchener *et al.*, 1965) that alkaline phosphatase activities in pregnancy are not normally distributed about a mean.

TABLE II.—Comparison Between the Heat-stable and Antibody-inactivated Percentages of Alkaline Phosphatase in 10 Individual Maternal Sera

Initials	Serum Alkaline Phosphatase		
	Total	Percentage Remaining after 30 minutes at 56° C.	Percentage Inactivated by Antibody
D.W.	11.4	36.8	22.8
S.P.	17.2	22.1	21.0
S.C.	13.6	61.8	41.2
R.H.	46.0	71.4	61.0
A.P.	22.0	43.6	40.0
V.P.	13.6	47.0	41.3
M.A.	15.2	27.6	34.3
E.M.	11.2	21.4	17.9
J.S.	20.2	43.5	40.6
M.B.	10.8	18.5	14.6
Mean of 10 ..	18.1	39.4	33.5

one-third after incubation with antiplacental phosphatase antibody, while the preheated maternal sera were inactivated to about 15% of the control values.

Table II shows the percentage of heat-stable alkaline phosphatase in each of the 10 maternal sera. This is compared with the percentage of alkaline phosphatase removed from each of the unheated sera by incubation with antiplacental phosphatase antibody. There is good agreement between the two sets of results.

DISCUSSION

The use of a specific anti-enzyme antibody is, in theory, the ideal method for the identification of an enzyme in a mixture of functionally similar enzymes (Cinader, 1963). Schlamowitz and Bodansky (1959) made use of such a method to identify bone as the tissue source of approximately 60% of the circulating alkaline phosphatase in normal subjects. Unfortunately different alkaline phosphatases cross-react immunologically with one another (Boyer, 1963), and the anti-enzyme used in these experiments partially inactivated intestinal phosphatase.

It is therefore theoretically possible that the antibody-inactivated material in the maternal circulation is of intestinal origin. However, the close correlation between heat stability and antibody sensitivity seen in Table II makes an intestinal origin for the maternal enzyme highly unlikely. Placental alkaline phosphatase is the only known human alkaline phosphatase stable at 56° C. (Neale *et al.*, 1965); intestinal alkaline phosphatase is inactivated at this temperature (Landau and Schlamowitz, 1961). It is therefore concluded that the heat-stable antibody-precipitated component of maternal serum alkaline phosphatase is derived from the placenta. It is this enzyme which is responsible for the elevation of serum alkaline phosphatase in pregnancy.

We have previously speculated about the reasons why a placental enzyme should be present in the maternal circulation and absent from the cord blood (McMaster *et al.*, 1964). One of us has since shown by electronmicroscopy that *E. coli* alkaline phosphatase is situated in an intermediate layer of the cell wall, just inside the outer membrane (Done *et al.*, 1965). If an analogous situation were to exist in the case of the human placenta it is reasonable to suppose that phosphatase would gain access to the maternal and not to the foetal circulation.

SUMMARY

Alkaline phosphatase in maternal sera at term is partially inactivated by an anti-human placental alkaline phosphatase antibody. No such inactivation is seen in sera from non-pregnant subjects or newborn infants. This provides strong evidence that serum alkaline phosphatase elevation in pregnancy is due to circulating placental enzyme.

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Medical Memoranda

Double Retrograde Intussusception of a Meckel's Diverticulum

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Since Meckel's description in 1809 an extensive literature has accumulated around the diverticulum and its many complications, including intussusception. Retrograde intussusceptions have been reported by Lewis (1936) and Mitchell (1936). Sanin (1961) describes a retrograde intussusception caused by a Meckel's diverticulum. Compound intussusceptions have also been reported, usually involving small and large bowel. D'Arcy Power (1899), Knaggs (1900), and Buckley (1919) have all described cases of double intussusception in which one intussusception was prograde, the other retrograde. Illyes and Gerlei (1957) have described a triple intussusception caused by a Meckel's diverticulum. There does not appear to be a previously reported case of a double retrograde intussusception caused by a Meckel's diverticulum.

CASE REPORT

The patient, aged 37, was a works driver. In childhood he had had an episode of severe abdominal pain which passed off spontaneously. One year before admission he had an episode of abdominal pain and vomiting which lasted for five days. Forty-eight hours before admission he passed two loose motions. Twelve hours later he developed a dull pain in the lower abdomen which later became severe and colicky, passing through to the back and radiating down both thighs. This continued for a further 24 hours until admission.

On examination the patient was in pain and vomiting faeculent material. His pulse was 86/min., temperature 97° F. (36.1° C.), and blood-pressure 90/55 mm. Hg. A tender mass was felt below

and to the left of the umbilicus. A succussion splash was heard in the lower part of the abdomen, and the bowel sounds were obstructive. On rectal examination a tender mass was felt in the pelvis on the left side.

The results of investigations were as follows: radiographs showed dilated loops of ileum; haemoglobin, 110%; blood urea, 80 mg./100 ml.; serum Na, 133 mEq/l.; serum K, 5.4 mEq/l.; serum chlorides, 94 mEq/l.

A pre-operative diagnosis of strangulated ileum, cause unknown, was made, and after rehydration with intravenous fluids the abdomen was opened through a right paramedian incision. A mass 18 cm. × 6 cm., was found in the lower abdomen and appeared to be a retrograde intussusception. There was considerable dilatation of the atonic proximal bowel. Because of the oedema of the mass and its doubtful viability reduction was not attempted but resection and end-to-end anastomosis performed. Dissection of the specimen revealed a second intussusception within the first, the inner one being gangrenous. The apex of the inner intussusception consisted of a necrotic Meckel's diverticulum. Subsequent histological examination showed no abnormal tissue in the diverticulum.

The patient made a good recovery, and when seen one month later was fit and back at work.

COMMENT

An intussusception is generally regarded as being formed by the intussusceptum invaginating the bowel wall and being pushed along the lumen of the bowel by peristaltic action, dragging the bowel to which it is attached behind it. Though many recently formed intussusceptions can be reduced manually chronic intussusceptions are often reduced with great difficulty as a result of adhesions formed between the intussusceptum and the intussusciptions. These adhesions also prevent the intussusceptum being driven further along the bowel. The intussusciptions and the intussusceptum then become a new mass

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 1.—Meckel's diverticulum adherent to bowel wall—proximal bowel being pushed over the mass. FIG. 2.—Retrograde intussusception has formed and become adherent to diverticulum and bowel wall. FIG. 3.—Second retrograde intussusception beginning to form. FIG. 4.—Double intussusception has now formed, leading to obstruction.