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UMBILICO-PLACENTAL ANTIGENS AND MALIGNANT NEOPLASMS*1

BY

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ABSTRACT

Serological reactions between anti-human placental tissue antiserum and 584 human sera from patients of various diseases and conditions have been tested using the micro-Ouchterlony method. Relatively high incidence of positive reaction was noted with cases of malignant tumours (57.5%), leukaemia (63.6%), pregnancy (77.8%), myocardial infarction (41.2%), and hepato-biliary diseases (43.3%). One of the most important and interesting characters of this test is the wide variety of neoplasms which showed a positive reaction. The active factor was located at β-area by immunoelectrophoresis, except in the case of pregnancy. Antigen analysis using a double diffusion method on agar plate showed quite a similarity between umbilical cord and placental tissue extracts, the active components of which were tentatively named umbilico-placental antigens.

INTRODUCTION

An example of recent progress in clinical application of cancer immunology is the discovery and utilization of α_1-fetoprotein[1]. This particular protein is, of course, closely related to the fetal tissue, and is also detectable in the serum of patients bearing hepatocellular or testicular carcinomas with high specificity. Besides, there are some other fetoproteins[2–4] which have been reported to be more or less immunologically common with abnormal components appearing in sera of patients mainly with neoplastic diseases. Thus, investigations for serum proteins which would be specific for limited kinds, or if possible, even wide range of malignant tumour cases are now being carried out everywhere, and their success is anxiously awaited because these factors might be quite useful for cancer diagnosis.

By way of extending our work on the subject of organ specificity and its possible alteration by malignant change[5], a fact was noted that there

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seemed to exist some specific antigen(s) which immunologically common between human malignant tumours and umbilico-placental tissue. Naturally a hypothesis was made that anti-umbilico-placental tissue antiserum might be used for cancer diagnosis, and a series of practical experiments with patients' sera of various kinds was carried out.

Materials and Methods

Rabbits were stimulated with freeze-pressed, crude homogenate of fresh human placenta or umbilical cord tissue obtained by cesarean section, mixed with Freund's complete adjuvant. Six to 18 weeks later, blood was collected from the auricular veins after additional, booster injections given one week before each bleeding. A small amount of sodium azide was added as a preservative after separation of the serum, which was absorbed with pooled, normal human serum repeatedly, until no precipitating lines were observed any longer on agar plate by the micro-Ouchterlony method.

Patients' sera were collected from central laboratories of several affiliated hospitals, serially numbered and kept frozen. Diseases of the patients depended on clinical diagnoses and, some of which were confirmed later by histological examinations at surgery or autopsy.

Antigen-antibody reaction between the antiserum and patients' sera was tested mainly by the micro-Ouchterlony method, and the result was read very carefully, usually 24 hours after preparation of the serum. Blind method was employed for the reading. Other kinds of double diffusion technique in agar using various forms of arrangement and immunoelectrophoresis were also adopted, especially for the purpose of antigen analysis.

Results

Data to be shown here as the results of our experiment were all obtained with anti-placental tissue antiserum, and that with anti-umbilical cord antiserum will be reported on another occasion, since no sufficient analysis has been made so far with the latter.

General incidence of positive reactions in various diseases

A total of 584 sera obtained from patients of various diseases (576 cases) and healthy men (8 cases) were tested. Table 1 shows the result, general incidence of positive reactions, which actually is a summary of 4 series of tests using 4 different lots of antisera. There seemed to be a little, but not serious, difference in the character of antiserum from lot to lot, which probably was due partly to non-uniformity of the titre of antibodies pro-

Table 1. Results of the test

Cases	No. of all case	Positive cases No. (%)	Negative cases
Malignant tumours	179	103 (57.5)	76
Malignant tumours, post-operative	21	7 (33.3)	14
Leukaemia	33	21 (63.6)	12
Benign tumours	11	1 (9.1)	10
Pregnancy	9	7 (77.8)	2
Myocardial infarction	17	7 (41.2)	10
Hepato-biliary diseases (benign)	60	26 (43.3)	34
Other diseases and healthy	254	29 (11.4)	225
Total	584		

duced in each rabbit and partly to different procedure of absorption including a lot of pooled normal human serum employed.

From this table, it is apparent that positive reaction occurred quite often in cases of malignant tumours. On the other hand, a limited number of some non-tumourous diseases or conditions, such as pregnancy, myocardial infraction, and hepato-biliary diseases were also frequently found to show positive reactions. There were some positive cases also in the group of miscellaneous diseases, but the incidence was not too high.

Analysis of the result with each group of diseases

1) Malignant tumours

A total of 179 cases of malignant tumours have been tested so far, among which 103 (57.5%) showed a positive reaction (Table 1). Table 2 shows detail of these cases, in which various kinds of malignant neoplasms are listed as the cases which more or less showed positive reactions. As far as tested, cases of carcinomas occurring from 19 different primary sites showed a positive reaction, among which ones from the stomach, colon, liver, pancreas, etc., gave rather a high incidence of positive reaction. In addition, multiple myeloma, malignant lymphoma, and soft part sarcoma are also listed here as members of sarcomas, among which the first two showed extremely high incidence of positive reaction.

The cases summarized here as malignant tumours in Tables 1 and 2 are the mixture of unselected cases of various kinds and stages of malignant solid tumours, for example, early cases such as carcinoma *in situ* of the uterine cervix, or early mucosal carcinoma of the stomach, far advanced cases in kachectic condition, and ones which had been given a large dose of oncostatics, etc. In general, cases in very early stage or after receiving intensive chemotherapy tend to be negative, while those of advanced cancer

Table 2. Cases of malignant tumours

Tumours	Incidence of positive reaction No. of cases/No. of all cases
Multiple myeloma	31/52
Gastric carcinoma	20/27
Intestinal carcinoma	9/15
Pulmonary carcinoma	6/13
Malignant lymphoma	6/11
Uterine carcinoma	1/9
Breast carcinoma	1/8
Hepatic carcinoma	6/6
Pancreatic carcinoma	3/5
Ovarian carcinoma	2/5
Oral cavity (except lingual) carcinoma	1/4
Bladder carcinoma, and miscellaneous sarcomas	2/3, each
Lingual, and bile duct carcinomas	1/2, each
Oesophageal, lower jaw, lacrimal gland, renal, vulvar, prostatic carcinomas, and seminoma	1/1, each
Maxillary carcinoma, and malignant thymoma	0/1, each
Malignant tumours of clinically unknown origin	4/5
Total	103/179

quite frequently show positive reaction, as far as the results with our present technique are concerned. Incidence of positive reaction in the group of post-operative cases (Table 1)—cases which had had surgical operation but no sign of recurrence appearing yet—is a little lower (33.3%) than that among non-operative cases (57.5%). However, the period between surgical operation and bleeding for serum collection varied very much from case to case, from several days to years, and it does not seem appropriate to discuss these cases together.

Twenty-one showed positive reaction among 33 cases of leukaemia of various types (63.6%) (Table 1), which could be divided into two groups of nearly equal size, non-treated and treated. Incidence of positive reactions in these respective groups were 11/13 in the former and 10/20 in the latter, which fact again suggests that the result of our test may often turn negative after treatment, especially during the period of remission.

2) Benign tumours

As for benign tumours, 11 cases have been tested so far and the result was all negative except for one case of uterine myoma (Table 1). Detail of these cases is shown in Table 3, some whose tumours were small in size, but tumour nodules of considerable size were evidenced in more than one-half of the cases. Taking these facts into consideration, our test seems to be little related with benign tumours.

Table 3. Cases of benign tumours

Serum No.	Age (yrs.)	Sex	Tumours
HS–0018	61	F	Meningioma (brain)
HS–0095	21	F	Fissural cyst? (upper jaw)
HS–0106	27	F	Haemangioma (brain)
HS–0120	49	F	Leiomyoma (uterus)
HS–0188		F	Leiomyoma (uterus)
HS–0289	30	M	Angimatosis (skin)
HS–0323		M	Haemangioma (skin)
HS–0362	23	M	Neurinoma (mediastinum)
HS–0462	49	M	Follicular adenoma (thyroid)
HS–1389*	29	F	Follicular adenoma (thyroid)
HS–1399	62	F	Leiomyoma (uterus)

* This case showed positive reaction.

3) Pregnancy

Incidence of positive reaction was very high (77.8%) in pregnancy, as shown in Table 1. As far as examined with nine cases listed here, antigenic substances seemed to appear in the serum as early as around the third month of pregnancy, which were still detected by the test at the time of nearly full term.

4) Myocardial infarction

General incidence of positive reaction in myocardial infarction was 41.2%, as far as 17 tested cases were concerned (Table 1). Among these, we had another chance to collect and test the serum from eight cases about two weeks after the first test which was performed shortly after attack in most cases. Comparative results were (+)→(+) three cases, (+)→(−) two cases, and (−)→(−) three cases.

5) Hepato-biliary diseases

Moderately high incidence of positive reaction (43.3%) was obtained also with cases of hepato-biliary diseases (Table 1). Detail of the cases is shown in Table 4, which apparently indicates that the highest incidence was seen in chronic hepatitis and liver cirrhosis. Cases of acute hepatitis or biliary system disorders (including obstructive jaundice) sometimes showed a positive reaction, but not too often.

Table 4. Cases of hepato-biliary diseases

Diseases	Incidence of positive reaction No. of cases/No. of all cases
Liver cirrhosis, and chronic hepatitis	18/30
Acute hepatitis	4/16
Gall bladder and bile duct diseases	4/14
Total	26/60

6) Other diseases and healthy condition

About 11% of positive results in incidence were obtained with cases of miscellaneous diseases (Table 1). None of the sera from healthy individuals (eight cases) showed a positive reaction. The actual number of cases which showed a positive reaction in this group was 29, and no specific tendency was noted with these cases. The antiserum was further absorbed with some of these 29 sera and, using a limited number of cases, the positive reaction with cancer patients' sera was confirmed to remain, although no precipitating line on agar plate was produced any longer with the sera which had been used for re-absorption. This fact suggests strongly that there is a possibility of still minimizing false-positive reactions of our test by improvement of the absorption technique.

There were some cases of the so-called autoimmune diseases included in the miscellaneous diseases and the test with these were all negative.

Attempted antigen analysis

Our absorbed antiserum usually produces two clear precipitating lines, one in α- and the other in β-area, when tested with crude placental extract by immunoelectrophoresis. The one in β-area is often separated into two, duplicated lines. With cord serum, only the one in β-area appears, while both can be seen when reaction is tested with sera obtained from pregnant women (Fig. 1). With sera from patients of various malignant diseases, precipitating lines appear usually at some place in β-area, but sometimes they are observed moving a little towards γ-area (Fig. 2). Usually, single line is produced in each case of malignant diseases, but double lines appear occasionally.

With double diffusion methods in agar, partial identity has been proved between the placental antigen(s) appearing in β-area and the ones found in the sera from cancer patients (Fig. 3). The cord- or fetus-serum, which unavoidably contaminates placental or umbilical cord tissue, is also tested as one of the most possible factors acting as the main source of the antigen(s). This shows only partial identity to either of placental extract or positive patients' sera, and our antigen(s) seems to contain some other important components. Precipitating lines obtained by respective cancer cases seem to fuse with each other on agar plate, but, no evidence has been obtained that they are exactly the same, single antigen (Fig. 4, 5, 6, 7). It is possible that there are more than one antigenic substances, all appearing in β-area, in the sera of cancer patients which are a little different from each other, but all have partial identity with the placental antigen(s).

Crude umbilical cord extract produces, with our absorbed antiserum,

a single precipitating line by immunoelectrophoresis in β-area which seems to be quite the same as the one observed when the reaction was made with the placental extract. Complete fusion of these two has been confirmed on agar plates (Fig. 8), and these umbilical and placental antigen(s) are definitely different from CRP (Fig. 9) or HCG (human chorionic gonadotropin) (Fig. 10), and the precipitating lines are stained with PAS reagent. Further analysis is now in progress.

Discussion

Considerably wide range of various diseases are practically covered in the results described above and, therefore, the general outline of our test using anti-placentral tissue antiserum seems to have been clarified. On the other hand, however, our antigen analysis has only just started and for this reason, this might be called a preliminary report of our work.

One of the pitfalls and the most difficult points in preparing the antiserum for this test seems to be in the method of absorption. Too much absorption results in unexpected, sudden decrease in the titre of antibodies as in the case of preparing organ-specific antiserum, and insufficient absorption naturally causes less specificity of the antiserum obtained. It is almost certain that future improvement of absorption technique as well as purification of antigenic substances for stimulation would increase specificity of this test.

The absorbed antiserum shows positive reactions with sera obtained from patients of many kinds of malignant neoplasms, and that is one of the most important and interesting characters of our test. At the same time, the antiserum reacts with patients' sera not only of malignant diseases, but also of pregnancy, myocardial infarction, and liver cirrhosis, as described above. Thus, it is apparent that our test is very closely related to malignant diseases of wide spectrum, but it cannot be called tumour-specific.

There is a possibility that the antigenic substance we are now handling and discussing may be composed of more than one antigens, and the question is whether it is identical to any of these reported by other workers. Up to date, several protein fractions which are more or less specific to malignant neoplasms have been discovered in the serum of cancer patients, some of which are internationally well known. α_1-fetoprotein[1] which seems to have been almost established and made available for practical use should not be identical to our factor, since the active component of ours always appears in the β-area in immunoelectrophoresis, except in the case of pregnancy. Carcinoembryonic antigens (CEA)[3] must be a different fac-

tor from ours, since CEA is said to be almost specific for gastro-intestinal carcinomas. β-fetoprotein reported by Nechaud, Economópoulos, and Uriel[4] does not either seem to be equal to ours when differences in reactions by pregnancy or hepato-biliary diseases are taken into consideration, although these two seem to be the most similar to each other of all. None of other β-proteins discussed in the review by Nechaud *et al.*[4] nor those reported intramurally in Japan have enough evidence to prove definite identity to our factor.

We should like to call our antigenic substance umbilico-placental antigens tentatively, until the detail of their character becomes clarified. They may be complexes of antigens, some of which are the so-called fetoproteins. If that is the case, it would be easier and morally better to use the umbilico-placental tissue for carrying out further work in this field than to use human fetus as the material. We expect that anti-umbilico-placental tissue antiserum should be useful for detecting malignant diseases by interaction with patients' sera, when some technical improvement and standardization can be made.

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Explanation of Figures

Figs. 1, 2. Immunoelectrophoresis
Reactions between our anti-placental tissue antiserum, absorbed, and several antigenic reagents. Two precipitating lines, one in α– and the other in β–areas are demonstrated with crude placental tissue extract (P–antigen) and serum from the pregnant, but onfy one in β–area is formed with sera from patients of various malignant tumours.

Fig. 3. Immuno-diffusion in agar
centre well; anti-placental tissue antiserum, adsorbed
peripheral wells; p—crude, placental tissue extract
p_c—serum from a cancer patient
Note some partial fusion of precipitating lines between the two, especially clearly seen around the lower left well.

Figs. 4, 5, 6, 7. Immuno-diffusion in agar
centre well; anti-placental tissue antiserum, absorbed
peripheral wells; (4) 20—serum from a patient of hepatocellular carcinoma (HS–20)
80—serum from a patient of pulmonary carcinoma (HS–80)
(5) 80—HS–80
475—serum from a patient of acute leukaemia (HS–475)
(6) 1, 3, 5—serum from a patient of ovarian carcinoma (HS–1412)
2, 4, 6—serum from a patient of oral cavity carcinoma (HS–1409)
(7) 1, 3, 5—HS–1409
2, 4, 6—serum from a patient of chronic myelogenous leukaemia (HS–1385)
Note almost complete fusion of precipitating lines between each other, in Figs. 4, 5, 6, and 7.

Fig. 8. Immuno-diffusion in agar
centre well; anti-placental tissue antiserum, absorbed
peripheral wells; 1, 3, 5—crude, placental tissue extract
2, 4, 6—crude, umbilical cord tissue extract
Note double precipitating lines are formed with placental extract, but single with umbilical cord extract which is identical to the internal line of the former (clearly seen around the well No. 3). The external line corresponds to α– and the internal to β–component of the P–antigen in Fig. 1.

Fig. 9. Immuno-diffusion in agar
centre well; serum from a patient of gastric carcinoma (HS–569)
peripheral wells; c—anti-CRP antiserum, absorbed
u—anti-placental tissue antiserum, absorbed
Note remarkable crossing of precipitating lines between each other.

Fig. 10. Immuno-diffusion in agar
centre well; serum from a patient of acute leukaemia (HS–475)
peripheral wells; g—anti-placental gonadotropin antiserum, absorbed
p—anti-placental tissue antiserum, absorbed
Note no fusion, but occasional crossing of precipitation lines between each other.

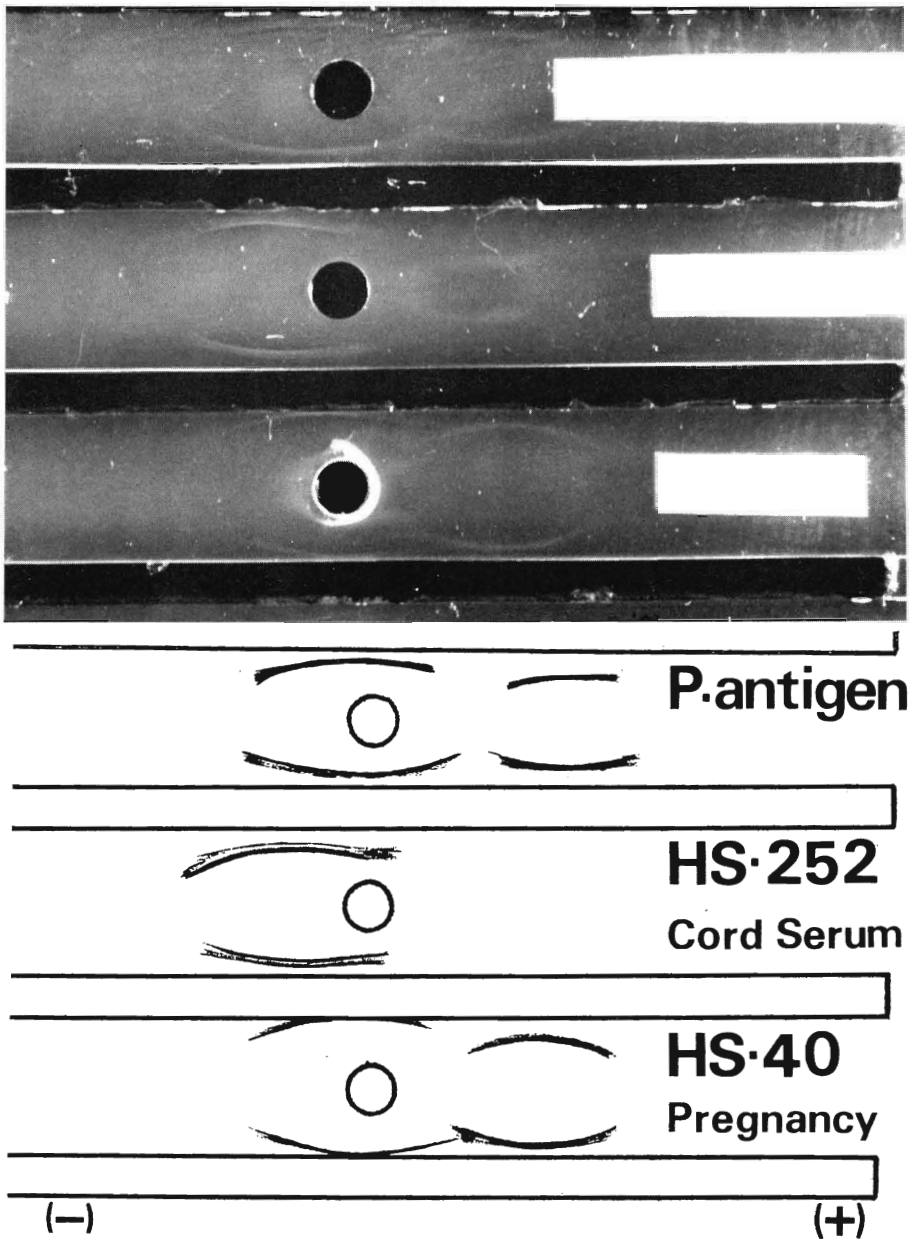

Fig. 1.

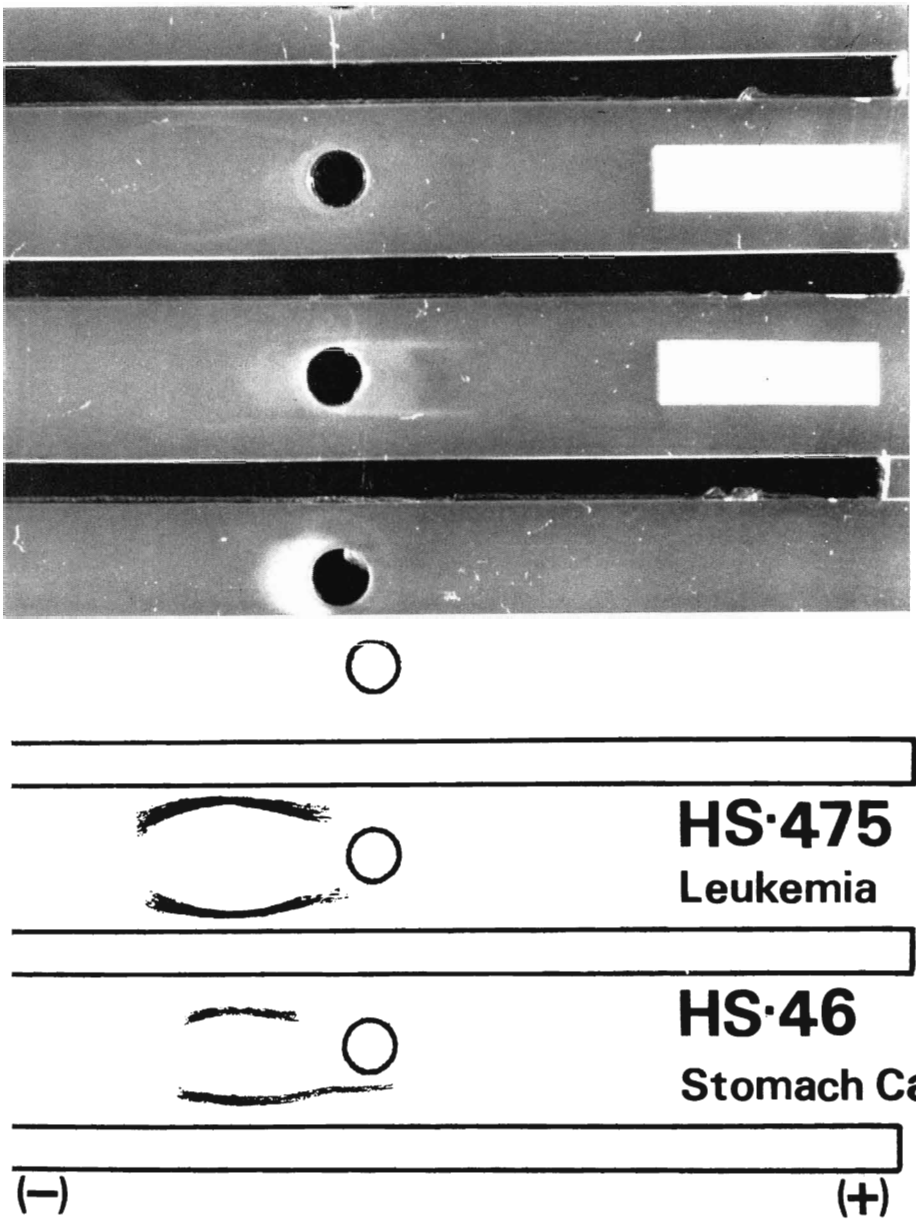

Fig. 2.

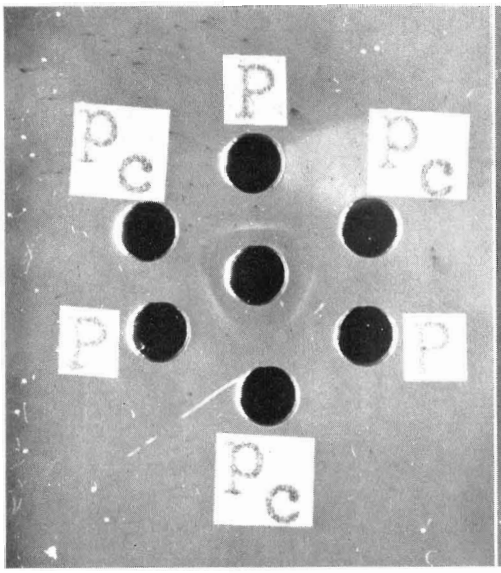

Fig. 3.

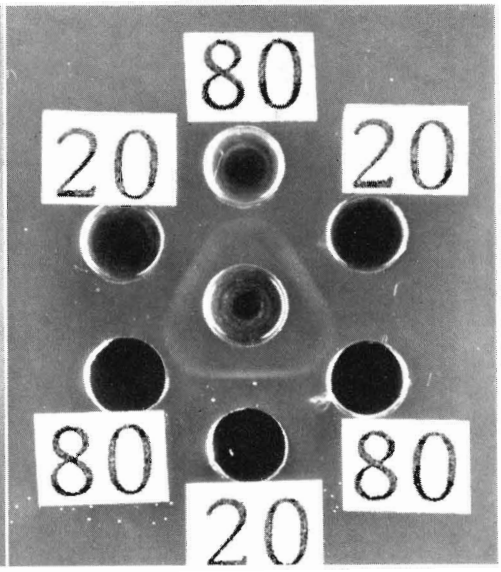

Fig. 4.

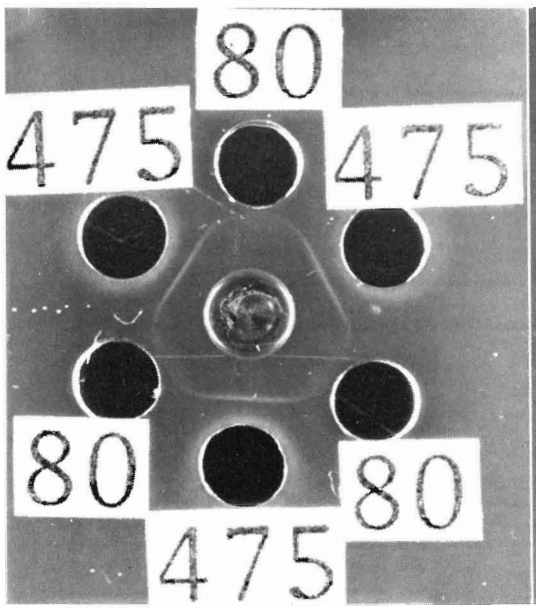

Fig. 5.

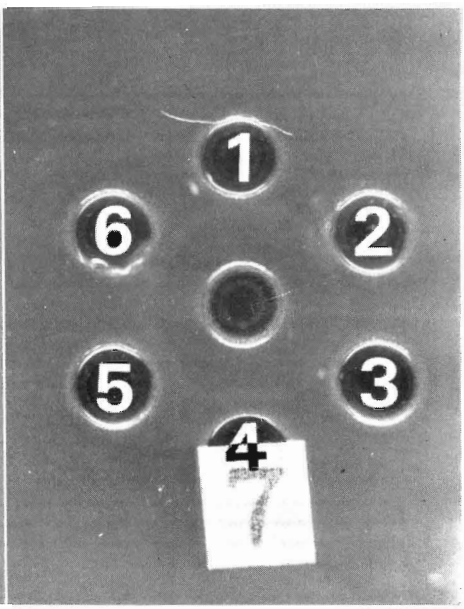

Fig. 6.

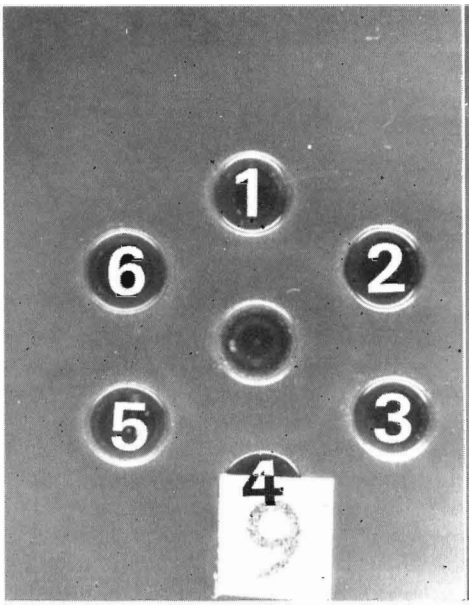

Fig. 7.

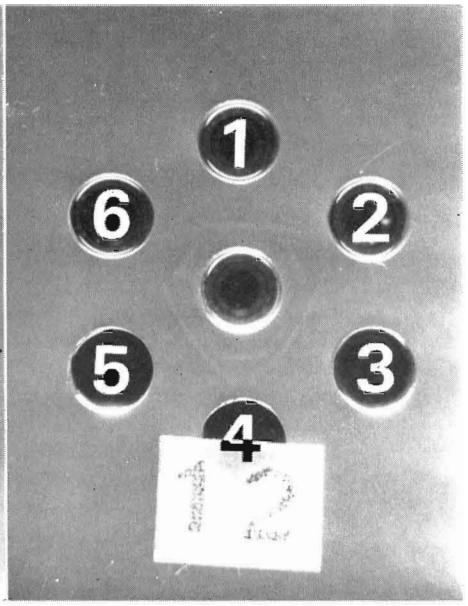

Fig. 8.

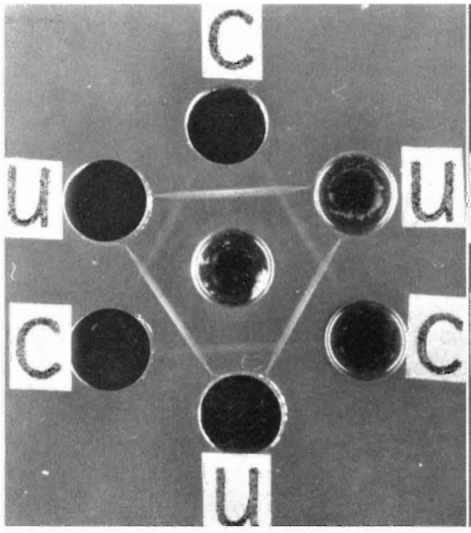

Fig. 9.

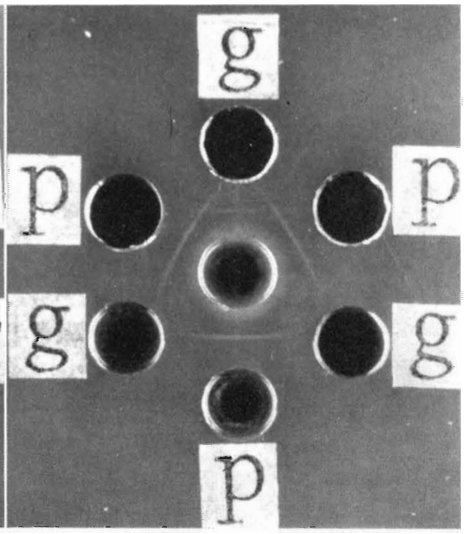

Fig. 10.