abnormality may be present and that a conservative policy may avoid caesarean section in these cases. The avoidance of prolonged labour is also important, and cases in labour longer than 24 hours call for reassessment if delivery is not imminent.

Even when an expectant policy is pursued, however, a high number of operative deliveries will still occur, so that these cases require the resources of a specialist maternity hospital. While this is in agreement with the views expressed by Macafee and Bancroft-Livingston, the problem of ante-partum meconium staining would seem to be more serious than they admit, since 19.2% of the cases in the present series required to be delivered by caesarean section or by forceps on account of foetal distress. It is not known whether serious asphyxia at birth is responsible for any impairment in subsequent mental ability, but there is much to be said in these cases for the view expressed by De Soldenhoff and Brill that the early signs of foetal distress must be observed and acted upon if maximum foetal salvage is to be secured.

Summary

Different views on the importance of meconium staining of the liquor amnii are presented, and a series of 125 cases in which ante-partum meconium staining was encountered is analysed.

An expectant policy is justified in most cases, but the incidence of foetal distress is greatly increased, as operative delivery was required on this account in 19.2% of cases.

Both the caesarean section rate and the forceps rate in the series were twice the hospital rate. These cases therefore require the resources of a specialist maternity hospital.

The importance of the recognition of the early signs of foetal distress in these cases is emphasized, as is also the avoidance of prolonged labour.

It is also noted that there is an increased incidence of an abnormal foetus in association with ante-partum meconium staining.

I thank Dr. R. A. Tennent for his advice and criticism in the preparation of this paper.

REFERENCES

- Burger, P., Milfort, J., and Tuchmann, L. (1956). *Bull. Féd. Soc. Gynec. Obstét. franç.*, **8**, 391.
Desmond, M. M., Moore, J., Lindley, J. E., and Brown, C. A. (1957). *Obstet. and Gynec.*, **9**, 91.
De Soldenhoff, R., and Brill, G. (1954). *Edinb. med. J.*, **61**, *Trans. Edinb. obstet. Soc.*, Session 106, p. 17.
FitzGerald, T. B., and McFarlane, C. N. (1955). *Brit. med. J.*, **2**, 358.
Lister, U. M., and Buchanan, M. F. G. (1957). *J. Obstet. Gynaec. Brit. Emp.*, **64**, 233.
Macafee, C. H. G., and Bancroft-Livingstone, G. (1958). *Ibid.*, **65**, 7.
Reiss, H. E. (1958). *Brit. med. J.*, **1**, 1394.
Shul, K. P., and Eastman, N. J. (1957). *J. Obstet. Gynaec. Brit. Emp.*, **64**, 227.
Walker, J. (1954). *Ibid.*, **61**, 162.

Mr. R. LLEWELYN DAVIES has been appointed to the London University chair of architecture tenable at University College from October 1, 1960. He was architect to, and later director of, Investigation into Functions and Design of Hospitals from 1948 to 1953. Since 1953 he has been director of the division for architectural studies of the Nuffield Foundation. Current or recently completed architectural works include a research laboratory at Cambridge and a hospital on Alderney (*University of London Gazette*, vol. 45, No. 9).

THE SCHULTZ-DALE ANAPHYLACTIC TEST FOR CARCINOMA ANTIGEN

BY

L. M. McEWEN, B.A.

From the Medical Research Council Department of Clinical Research, University College Hospital Medical School, London

Makari and Huck (1955) and Makari (1958) have reported that a carcinoma antigen could be detected in the blood plasma of carcinoma patients. To detect the antigen they used the anaphylactic response of uteri from appropriately sensitized guinea-pigs described by Schultz (1910) and Dale (1913). Burrows (1958) has confirmed these findings. The work described below was an attempt to repeat these experiments.

Method

Human carcinomata were obtained at operation, and extracts were prepared as described by Makari and Huck (1955) and Makari (1956, personal communication). Homogenates were made in a rotary blender run at approximately 15,000 r.p.m. for 30 minutes, the blending container being packed in ice. They were then centrifuged at 3,000 r.p.m. (950 g) to remove debris. The supernatant from this centrifugation was recentrifuged at 10,000 r.p.m. (10,200 g) for 45 minutes, the precipitate being collected and stored at -20°C . This was the antigen to which guinea-pigs were to be sensitized.

Following the method of Makari and Huck, adjuvants were used with the object of increasing the sensitizing power of the antigen. The following modification of Freund's adjuvant was used:

"Bayol F"	8.5 ml.
"Arlacel A" special	1.5 ml.
<i>Mycobacterium tuberculosis</i>	5 mg. (steam-killed desiccated human bacilli)

These ingredients were mixed, an equal volume of a 20% suspension of antigen was added, and an emulsion was made in a glass blender.

Young virgin female guinea-pigs were given 1 ml. each of the emulsion by multiple subcutaneous inoculation. They were used for sensitivity tests between three and six weeks after inoculation.

Blood plasma for testing was obtained from patients with carcinoma and from normal subjects by venepuncture. A nylon syringe was used, and subsequently the blood and plasma were handled entirely in "polythene" or in siliconed glassware. Dry heparin was added and the blood cells were removed by centrifugation as soon as possible. The plasma was stored at $+4^{\circ}\text{C}$. for one to four days and was then divided into small samples, each sufficient for one test, and frozen to -20°C . It was allowed to thaw at room temperature just before it was needed. In the last two series of experiments the plasma was dialysed in "cellophane" against normal saline at $+4^{\circ}\text{C}$. for 24 hours, and soya bean trypsin inhibitor (S.T.I.) was added at a concentration of 1 mg./ml. before the plasma was frozen.

Blood plasma for desensitization of the uterus preparations was obtained from the human blood bank. Group O rhesus-positive blood was used. After removing the blood cells this plasma was also frozen

in small portions. Any plasma tinted pink through haemolysis was discarded.

For the anaphylactic test, a horn, or part of a horn, of the uterus of a sensitized guinea-pig was mounted on an isolated organ bath of 2 ml. capacity, filled with half-calcium Tyrode's solution (composition: NaCl 8 g./l.; KCl, 0.2 g./l.; CaCl₂, 0.1 g./l.; MgCl₂, 0.01 g./l.; NaHPO₄, 0.05 g./l.; NaHCO₃, 1 g./l.; dextrose, 1 g./l.). The solution was oxygenated and kept at 37° C.

As control, an unsensitized horn was similarly mounted and treated in exactly the same way as the sensitized one throughout the test. Movements of the muscle were recorded by levers writing on a smoked drum. The experiment was duplicated, using two parts of each uterus in separate baths. The horns were first desensitized to the normal constituents of human plasma by the group O plasma. When the first dose of 0.1 ml. of plasma was given, a large contraction of the sensitized preparation always occurred within half a minute.

After washing out with fresh solution, a successive dose usually produced less response, and this was repeated until no further response was obtained. In later experiments this process was curtailed by giving a larger initial dose (0.5 ml.) of the desensitizing plasma.

When desensitization was complete, 0.1 ml. of the plasma to be tested was added to the bath. A contraction of the sensitized horn of one-fifth or more of maximal size, and occurring up to five minutes after the addition of the test plasma, was said by Makari and Huck to indicate the presence of carcinoma antigen in the plasma. If the unsensitized preparation also contracted, it was said that an unwanted contracting substance had formed in the plasma.

Results

During the four series of tests 42 guinea-pigs were sensitized, using antigen from a pool of five mammary carcinomata and from five separate carcinomata of colon or rectum. Freeze-dried pooled antigen from six other carcinomata of colon or rectum were also used. Of the 64 preparations of sensitized uterine muscle set up, 13 failed to become satisfactorily desensitized to group O plasma and were excluded from the tests.

In the first two series of experiments plasma samples were collected from seven carcinoma patients. Four of these samples were pooled, but the other three were used separately. These plasma samples produced contractions in 19 out of 34 sensitized preparations and in 13 out of 28 control preparations. Although the sensitized preparations contracted more often than the control preparations, the difference is not statistically significant ($\chi^2=0.26$; for $n=1$). The pooled plasma and one of the separate samples contracted the preparations with which they were tested less frequently than the other two samples, but every plasma contracted some of both the sensitized and the control preparations. Plasma was also taken from one healthy subject. This contracted five out of seven preparations with which it was tested.

In the second two series of experiments, plasma was taken from three carcinoma patients and three normal subjects, and unwanted contracting agents were eliminated by dialysis and the addition of S.T.I. Plasma

from the carcinoma patients was tested with 17 sensitized preparations and produced no contractions. Plasma from a normal subject was also used with each of these preparations, and produced no response. The dialysed plasmas had no effect on any of six unsensitized preparations. However, a portion of one of the samples from a normal subject was treated as in the earlier experiments, and was found to possess strong contracting activity with both sensitized and control preparations.

There was great variation between the sensitivities of different uteri and even of the different parts of the same uterus. Different parts of the same uterus gave conflicting results with portions of the same plasma sample in 12 experiments. Further, on no fewer than six occasions the unsensitized preparation contracted in response to a test dose of plasma which had no effect on the sensitized preparation for which it was the control.

It was a further difficulty that the uterine muscle often underwent spontaneous contractions, and these were sometimes large and irregular even though the half-calcium Tyrode's solution recommended by Dale was used throughout the experiments. The contractility of the preparation as evidenced by this spontaneous activity, and by the responses to desensitizing doses of group O plasma, did not differ appreciably between experiments conducted in January and in August.

Discussion

Though reproducing as faithfully as possible the conditions laid down by Makari and Huck, it has not been possible to demonstrate carcinoma antigen in the plasma of patients with carcinoma.

Guinea-pigs were sensitized using antigen derived from a number of carcinomata of rectum and colon, which were said by Makari and Huck to be a good source of antigen, and from several carcinomata of the breast, which Burrows considered equally satisfactory. Since all the inoculated guinea-pigs appeared to become strongly sensitized to the normal constituents of human plasma, it seems that a fault in the technique of sensitization was unlikely to be responsible for our failure to elicit an anaphylactic response to carcinoma antigen.

The appearance of unwanted contracting agents in the plasma samples constituted a major difficulty. Makari and Huck stated that they could eliminate these substances from their samples of plasma by handling them entirely in "non-wetting" containers and by storage for 24 hours at +4° C. before freezing. Burrows only found it necessary to prevent haemolysis. However, in our experience, blood plasma taken from both carcinoma patients and normal subjects which had been handled with these precautions often contained substances that caused the muscle of the guinea-pig uterus to contract, whether sensitized to carcinoma or not.

The method of desensitization used by Makari and Huck and by Burrows was found by them to require repeated doses of group O plasma, and this was confirmed in the present experiments. In contrast, the uteri of three guinea-pigs highly sensitized to egg albumen were found to be completely desensitized to this antigen after a single dose only just sufficient to

elicit an anaphylactic response. This is in accord with the original work of Dale (1913), who showed that only when uterine muscle was too thick for rapid penetration by the antigen could a second dose produce even a weak anaphylactic response. In the present investigation the thickness of the muscle was unlikely to be important, since the guinea-pigs were young virgins and their uteri were small. It does not seem likely that the sensitivity elicited to the normal antigens of human plasma by following the method of Makari and Huck would be materially different from the sensitivity which can be elicited to their antigens. It is possible, therefore, that the repeated contractions produced by successive doses of group O plasma were not purely anaphylactic responses, and that the apparent desensitization which was achieved was not due to anaphylactic desensitization.

Besides the contracting substances like histamine and 5-hydroxytryptamine likely to be formed in blood and plasma which is being handled, Schachter (1956) has described a slow contracting effect which manifests itself one-half to three minutes after serum or plasma has been diluted in Tyrode's solution. He considered that a substance was formed by an enzyme system which was activated on dilution. This phenomenon may well have accounted for many of the contractions seen in the present experiments, since 60% of them were delayed for half a minute or longer. The method described by Makari and Huck was considered by Schachter (1956, personal communication) to be suitable for the demonstration of this delayed activity, since the dilution of plasma in the Tyrode's solution was of the correct order, and a contraction which was delayed even for five minutes was to be accepted as a positive result. Unfortunately, Schachter also reported that the delayed effect in human plasma is unpredictable and not easily inhibited. However, it was thought that S.T.I. at a final concentration of 50 $\mu\text{g./ml.}$ might suppress the enzyme system sufficiently for the purposes of the experiment.

This concentration of S.T.I. has been shown by Mongar (1956) not to inhibit appreciably the liberation of histamine from guinea-pig lung by anaphylaxis. Schachter also advises dialysis to ensure the elimination of small-molecule contracting substances formed in the plasma during its early handling. Burrows and Neill (1958) have stated that dialysis did not remove the antigenic fraction from his samples of serum.

Although these precautions were effective in eliminating contracting activity from the plasma samples, still no carcinoma antigen could be detected.

In addition to the difficulties experienced, due to the frequent detection of contracting agents in the plasma samples, the responses of the uterine muscle preparations to these pharmacological stimuli were erratic. Makari and Huck, and Burrows, considered the reactions of a single sensitized preparation, with its control, to be adequate to demonstrate the presence of carcinoma antigen in a sample of plasma. But we found that the unpredictable sensitivity of the preparations to other stimuli, and their frequent high level of spontaneous activity, made it impossible to attach this significance to the result of a single experiment.

The findings in the present experiments differed from those of Makari and Huck and of Burrows on three other points. (1) Burrows reported that a large

proportion of his inoculated guinea-pigs failed to become sensitive, even to the normal group O plasma. (2) He found it necessary to give the animals oestrogens during the winter to ensure that their uteri would be contractile. Although young guinea-pigs were used, neither of these difficulties was met with in our experiments. (3) Neither Makari and Huck nor Burrows detected the slow contracting substance described by Schachter. Since they took no precautions which would have eliminated it, the sensitivity of their preparations to this stimulus was probably low.

Summary

An attempt has been made to repeat the experiments described by Makari and Huck (1955) for the detection of carcinoma antigen in the plasma of patients suffering from carcinoma.

Carcinoma antigen was not demonstrated.

It was found that the precautions advised by Makari and Huck did not prevent the frequent appearance of contracting activity in the plasma samples, probably due to pharmacological agents. The conditions of the experiment were suitable for the demonstration of the slow contracting effect described by Schachter (1956).

This difficulty was avoided by dialysis of the plasma samples and the use of soya bean trypsin inhibitor.

I thank Dr. J. L. Mongar, who kindly allowed me to use his laboratory in the Department of Pharmacology, University College, and has given me much help and encouragement; and Dr. M. Schachter for his criticism and advice. My thanks are also due to a number of my student colleagues of University College Hospital who helped greatly during the earlier experiments. I am indebted to the surgeons at University College Hospital, the Princess Beatrice Hospital, the Royal Marsden Hospital, St. Mary's Hospital, St. Thomas's Hospital, and the Westminster Hospital, who have provided me with carcinomata.

REFERENCES

- Burrows, D. (1958). *Brit. med. J.*, **1**, 368.
 — and Neill, D. W. *Ibid.*, **1**, 370.
 Dale, H. H. (1913). *J. Pharmacol.*, **4**, 167.
 Makari, J. G. (1958). *Brit. med. J.*, **2**, 358.
 — and Huck, M. G. (1955). *Ibid.*, **2**, 1291.
 Mongar, J. L. (1956). In *Histamine: Ciba Foundation Symposium*, p. 444. Churchill, London.
 Schachter, M. (1956). *Brit. J. Pharmacol.*, **11**, 111.
 Schultz, W. H. (1910). *J. Pharmacol.*, **2**, 221.

Factory accidents reported in 1958 fell by 4% over the previous year to their lowest level for 23 years, according to the Annual Report of the Chief Inspector of Factories. The number reported fell from 174,713 in 1957 to 167,697, although fatal accidents rose slightly from 651 to 665. Although there was an increase in the number of fatal accidents in 1958 compared with 1957, the reduction of about 4% in the total number is a continuation of the downward trend in these figures during the last three years. The Chief Inspector regretted that in building operations and works of engineering construction there had been an appreciable rise in the accident figures. On building operations the total number of accidents in 1958 was 15,017, of which 207 were fatal, compared with 14,568 and 156 in 1957. On works of engineering construction the total for 1958 was 2,329 with 51 fatalities, and the corresponding figures for the previous year were 1,924 and 31. "Since 'here was no appreciable increase in the amount of work of this type in 1958 over that undertaken in 1957,' the Chief Inspector remarks, 'the figures can only be regarded as disappointing and clearly show the need for more positive action by the industries concerned.'" (H.M.S.O., Cmnd. 810, price 5s. 6d. net.)