TABLE II.—*Results of Examination of Sputum and of Hay Samples for Fungi*

Case No.	Sputum	Hay Samples
2	No fungi—a few yeasts only	Mixed assortment—*Aspergillus, Penicillium, Mucor, Absidia, Cladosporium.* Fewer fungi and more bacteria
6	A few yeasts only	Mixed assortment—*Aspergillus, Penicillium, Mucor, Absidia, Cladosporium*
8	Light growth of fungi and yeasts	Mixed assortment—*Aspergillus, Penicillium, Mucor, Absidia, Cladosporium*
9	No fungi	Mixed assortment—*Aspergillus, Penicillium, Mucor, Absidia, Cladosporium.* Relatively large number of *Aspergillus fumigatus* colonies

confirm the presence of an infecting agent (Table II). In other cases no fungi and only a few yeasts were found. Specimens of the offending hay were examined for fungi in four cases, but no fungus predominated and all samples showed the usual wide assortment of moulds. The specimen of hay in Case 9 differed from the others in that relatively large numbers of colonies of *Aspergillus fumigatus* were present, only one colony of this species being recognized in the other three specimens. The patient in Case 9 did not show any deviation from the usual clinical picture and *x*-ray appearances, neither were any fungi found in his sputum. The hay specimen in Case 2, however, differed in that many more bacteria developed on the culture plates and fewer fungi were present. The patient in this case had a slower convalescence, and it may be that the course of his disease was modified by secondary or concomitant bacterial infection. In this case no fungi were found in the sputum.

There appeared to be no relationship between the fungi found in the sputum and those grown from the corresponding specimen of hay in the cases examined. Although the cases fulfilled Fawcitt's criteria for the diagnosis of bronchomycosis, and although they had the same occupational history, the same mode of infection and of onset, the same clinical findings, with dyspnoea out of all proportion to other symptoms, similar radiological appearances, and a tendency to recover after administration of large doses of potassium iodide, there was no evidence that the same infecting agent was responsible in each case or that there was any growth of the organism within the bronchial tree. Therefore, in our opinion, the condition was not a true bronchomycosis.

Intradermal tests for allergic reactions were not performed owing to the difficulties in making arrangements in a widely scattered hilly area. In any case, Fuller's experience of the intradermal injection of extracts of both good and mouldy hay did not prove that sensitization was due to anything more than the presence of a histamine-like substance in hay.

It is our impression that in the development of farmer's lung there is such a high concentration of hay dust, consisting largely of mould spores, in the lower respiratory tract that the terminal bronchioles become literally choked. In addition to this purely mechanical interference with normal respiratory function it is probable that there is also a mild inflammatory response by the bronchial epithelium. Mere mechanical action and multiple minute traumata to the bronchial epithelium cannot, however, be the only factors involved, as several patients declared they had never had anything wrong with their chests before and were well accustomed to working in normal dust from good hay. Furthermore, relatives and others working under the same conditions were unaffected by the mouldy hay. The presence of fungi in the dust is undoubtedly the chief factor, but in the absence of histological proof their mode of action is uncertain. There is no evidence to explain why only certain persons are susceptible and others are not. Any tissue changes which may occur are usually of a temporary nature and clear up quickly once the invader is removed. The effect of potassium iodide may be twofold: (1) by a specific action as in actinomycosis; and (2) by increasing the flow of bronchial secretion due to stimulation of the epithelial cells it enables the dust particles and spores to be washed out of the bronchioles.

It is concluded that exposure to the dust arising from mouldy hay or corn is responsible for the development in susceptible persons of a distressing respiratory condition known as farmer's lung. In our experience hill farmers as a class are usually very reluctant to seek medical advice, either because of the inaccessibility of their farms or owing to pressure of work; therefore it is reasonable to suppose that many cases, especially when of mild degree, are not recorded at all. Failure to see and recognize the condition and to take the necessary precautions to avoid repeated attacks may lead to irreversible changes in the lungs and permanent incapacity.

Summary

Brief reference is made to the literature of the condition known as farmer's lung. A further ten cases are reported which occurred in epidemic form in 1955 in a hilly district of mid-Wales. Three sporadic cases seen earlier in which the diagnosis of farmer's lung was not readily apparent are also described. Evidence is presented in support of the view that the condition is not a true bronchomycosis.

We wish to acknowledge our indebtedness to Dr. G. C. Ainsworth, mycologist, University College of Exeter, for his report on the specimen of hay included in Table II.

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EXPERIENCE WITH ABO AND Rh BLOOD-GROUPING CARDS (ELDON CARDS)

BY

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After some years' experimental and practical work with cards for simultaneous ABO and rhesus determinations (Eldon, 1955; Jordal, 1955), these cards were put into large-scale industrial production by Nordisk Insulinlaboratorium. The technical process ensured uniformity of all the cards of each series, each card being packed in a moisture-proof envelope.

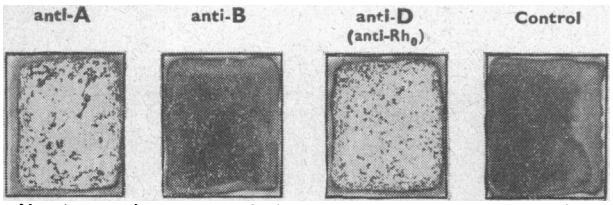
In February, 1955, the cards were released for use in hospitals and by practitioners, and in the ensuing year 64,000 cards were distributed in Denmark, of which at least 60,000 are estimated to have been employed up to the present.

The Card and Method of Use

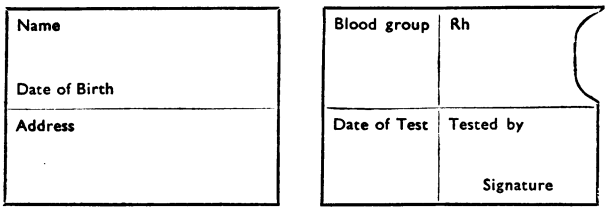
The card has four panels covered with regenerated cellulose ("cellophane"). In each panel 0.06 ml. (1 minim) of reagent is allowed to dry (100 ml. of reagent is composed of 10 ml. of serum, 90 ml. of 6% dextran, and two drops of 5% heparin solution). Anti-A and anti-B for ABO grouping and anti-D (anti-Rh_0) for Rh grouping are found in that order in the panels (see Fig.). The serum in the control panel does not contain antibodies.

Moulded plastic sticks fashioned with a flat terminal surface for measuring capillary blood are delivered with the cards, together with a controlled dropper pipette.

The blood groupings are performed direct on to the cards at room temperature. The reagents are either dissolved in water, after which capillary blood is added (technique 1), or in a saline suspension of blood cells (technique 2).



Negative reaction with anti-D demonstrates that the person tested is Rh-negative as recipient (but not necessarily as donor).



A card used with technique 1.

Technique 1

Technique 1 is intended for the grouping of persons present.

A drop of tap-water is added to each reagent on the card with the dropper pipette, which is held perpendicular. The reagents are dissolved by stirring with the flat end of the plastic stick, dabbing off in the panel the residue of liquid adhering to the stick. The stick must be thoroughly wiped after each stirring, and it is advisable either to dip the stick in water and wipe it once more or to make a control stirring in the control panel and then wipe the stick again.

A large drop of capillary blood freshly expressed from the ear lobe is now touched by the flat end of the stick and a hemisphere of blood is removed. The blood is mixed with the dissolved anti-A reagent on the card, after which the mixture is spread out over the whole panel. The stick is thoroughly wiped as described. The same procedure is followed in measuring blood for the other panels.

An interval of one minute allows the antibodies to attach themselves to the blood cells. This time should be used in filling out the card according to the information given by the patient. The card is then tilted slowly to all sides for three minutes and is held so far as is possible perpendicularly for 10 to 15 seconds at each end while the mixtures slide from border to border in the panels. The result is then read (see Fig.) and the card allowed to dry.

Technique 2

Technique 2 is intended for investigation of blood specimens in laboratories or blood banks, and particularly of the blood in the pilot tubes attached to the bottles containing donor blood. An anticoagulant should be added to the blood sample—for example, heparin, but not acid-citrate/dextrose solution, which inhibits the agglutinations. The blood cells are sedimented by allowing the sample to stand for six to eighteen hours at 4° C.: in urgent cases by centrifugation. The pilot tubes should not, however, be removed from their respective bottles, and, in order not to counteract the sedimentation, they should be placed vertically.

In carrying out the grouping, the plasma is drawn carefully from the specimen and the pipette is rinsed. Twelve drops of saline solution are mixed with three (after centrifugation only two) drops of the blood cells. One large drop of the mixture is placed on each panel of the card while holding the pipette at an angle of 45 degrees. Each panel is now stirred until all the reagent is dissolved and mixed with the suspension, which is then spread completely over the panel. The stick must be thoroughly wiped after each stirring as described. After an interval of one minute the card is tilted back and forth as in technique 1, but only for one minute. The results are read and the card is allowed to dry.

The following account deals with cases where results were doubtful or errors occurred. The cases are summarized in the Table. I assume that all such cases have come to my knowledge.

Errors

There were four ABO grouping errors. One of them was due to determination of a wrong sample and another to incorrect recording.

A bottle of A-donor blood was labelled O because the pilot test-tube had been removed from the bottle during the investigation and exchanged with an O-blood sample. The error was detected by a compatibility test.

Errors and Doubtful Cases, Reported from Approximately 60,000 ABO and Rh Groupings by Cards in Denmark

No. of Cases	Correct Group	Registered as	Technique	Cause of Error (or Doubtful Reaction)	Error Revealed By
			ABO Groupings		
Errors 1	A	O	2	Wrong sample	Compatibility test
1	A	B	2	Incorrect recording	Checking card
1	A_3	O	2	Emergency groupings with too heavy suspensions	Routine grouping by card
1	A_3	O	2		Control test
Doubtful cases 1	A_2B	B? AB?	2	Incomplete dissolution of reagent	Appearance of reactions on cards
3	A	A? (AB?)	1	Too high temperature during the manufacturing process	
			Rh_0 (D) Grouping		
Errors 1	Pos.	Neg.	1	Drying up before tilting (very slow performance)	Control test
3	,,	,,	1	Too much water added	,, tests
Doubtful cases 6	,,	Neg.? Pos.?	1	,, ,, ,, ,,	Appearance of reactions on cards
			Cases in which Neither the ABO Nor the Rh Group could be Read		
3		?	2	Haemolysis after freezing	Appearance of reactions on cards
2	A pos.	?	1	Protein disorders ? (Diseases in the biliary tract)	Agglutination in all panels, including the control panel
1	O ,,	?	2	Autoagglutinins ? (Pernicious anaemia)	
2	A ,,	?	2	Autoagglutinins (Idiopathic acquired haemolytic anaemia)	
2	A ,, O ,,	?	2	Bacterial action on the cells (Infectious secondary haemolytic anaemia)	

B blood was recorded on a card that showed A. The following day a compatibility test with B-donor blood was performed, without the error being revealed, however. It was not until the card was checked immediately before the transfusion that this error was disclosed.

The other two ABO grouping errors consisted in the weak A_3 factor remaining undetected by technique 2.

An emergency determination of umbilical-cord blood showed O for an A_3 child owing to the suspension used being of a very high density. Also, the child's A_3 factor was even weaker than that of an adult. The error was detected the following day, when the emergency determination was checked—by way of routine—through another grouping on cards.

Another A_3 patient was grouped as O by an emergency determination. A high-density suspension had been used in this case too. The error was detected by a control test performed at a specialized laboratory.

Only a small portion of the red cells of A_3 blood are agglutinable by anti-A: in dense suspensions the agglutinates are covered by the unagglutinated cells. In the two emergency determinations the suspensions became extremely dense because the samples were centrifuged at high speed before the preparation of the suspensions of—as then

prescribed—3 drops of cells in 12 drops of saline. On account of this result with A_3, the directions were accordingly revised, so that only two drops of cells are required if the blood sample has been centrifuged.

Clinically, the two errors with A_3 blood are insignificant; no complications have been reported—so far as I am aware—through A_3 recipients getting O blood; nor have any complications been caused even by O patients receiving A_3 blood. The incidence of A_3 in Denmark is reported to be approximately 1 per 1,000 (Andresen, 1952).

There were four rhesus errors, Rh-positive patients being grouped as Rh-negative by technique 1. In none of the tests in question was the recommended procedure applied.

One investigator had spent such a long time in stirring the mixtures that they had dried so much as to remain immovable in the panels when tilted. Hence the blood cells could not contact each other and agglutinate.

In the other three cases too much water had been added in order to dissolve the reagents. One investigator had slanted the pipette so much as to produce a water drop twice as big as that formed when the pipette is held exactly vertical. Another investigator had held the pipette so close to the card that the drop did not fall freely, and consequently was uncontrollable and too big. The third investigator had deliberately added too much water. Moreover, in one of these tests far too little blood had been used, giving the panels a faint pink colour only. These errors were disclosed at the laboratories.

Doubtful Cases

Uncertain ABO reactions, occasioning no actual errors, occurred in one case in the anti-A panel and in three cases in the anti-B panel.

A doctor, making her first independent blood grouping on cards by technique 2, obtained only a faint agglutination in the anti-A panel from an emergency determination on an A_2B patient carried out at night. The reason was that she had dissolved only a small part of the anti-A reagent. She did not test the patient's serum against known A and B cells—which would have been futile unless the A cells were A_2—since the patient's serum contained strong anti-A_1. However, she repeated the typing with the same technique and result, gave the patient B blood, and postponed the final blood grouping until the next morning.

In three cases, at one hospital, technique 1 brought out small-grained agglutination, of unreliable appearance, of A blood in the anti-B panel. There was reason to believe that this might be ascribable to excessive heat during the drying of the cards. The technique of the drying process was accordingly altered.

Uncertain Rh reactions occurred in six patients when technique 1 was incorrectly applied.

All the patients were of the normal Rh-positive type and not Du. When the results were read, no or only faint agglutination showed in the anti-D panel; but, since the anti-D-panel agglutination was of normal appearance two to thirty minutes later, the investigations were repeated. In all these tests too much water had been added on the panels, as was also the case with the Rh errors previously mentioned.

ABO as well as Rh results of three simultaneous tests, by technique 2, were uncertain, the blood cells giving the appearance of being haemolysed in the panels.

This phenomenon proved to be traceable to the complete haemolysis of the blood cells before the grouping, the samples having been frozen during storage in a refrigerator after sedimentation.

Seven cards could not be read owing to agglutination in all panels, including the control panel. It was demonstrated that in all these cases the blood agglutinated in all panels by technique 1. In two cases of diseases in the biliary tract the grouping was performable by technique 2. In three cases with autoagglutinins the cells could first be grouped by technique 2 after washing the cells at a raised temperature. In two cases of infectious haemolytic anaemia the cells could not be grouped on cards even after washing them at 56° C. Application of the classic saline technique involved no difficulties except in one of the two latter cases,* in which the O Rh-positive cells agglutinated in some anti-A and anti-B sera, but not in others.

*The other case was investigated by Dr. P. Ejby Poulsen and will be reported by him elsewhere.

Summary

In Denmark, during a period of one year, approximately 60,000 ABO and rhesus groupings were made on cards. The following errors have been reported: two technical ABO errors, four technical Rh errors, and two errors of recording. The ABO errors consisted in the rare and weak A_3 factor escaping notice as regards two A_3 patients grouped by technique 2, by which the reagents are dissolved in a blood-cell suspension; the errors were caused by the suspensions being too dense. The Rh errors consisted in four Rh-positive patients being grouped as Rh-negative by technique 1, by which the reagents are dissolved in plain tap-water before addition of capillary blood; the errors were caused by incorrect quantities of liquid. In 20 cases—that is, about 0.3 per 1,000—the results were doubtful or could not be read; in 13 of these cases this was attributable to misapplied technique, and in the remaining seven cases to the blood cells being abnormally agglutinable.

The results give the impression that to be of use the method must be closely adhered to, and the directions followed carefully.

I wish to acknowledge the help of grants from the King Christian X Foundation, the Simon Fougner Hartmann Family Foundation, and the Vera and Carl Michaelsen Foundation.

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RECENT STATISTICS OF CARBON MONOXIDE POISONING

REPORT TO THE MEDICAL RESEARCH COUNCIL

BY

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In a letter to the *British Medical Journal* of February 11, 1956 (p. 347), Dr. H. L. Marriott wrote that at about the end of 1952 and during 1953 "CO_2/O_2 mixtures were withdrawn from the first-aid services of the country, and only pure oxygen allowed, on advice by the Medical Research Council's Committee for Research on Breathing Apparatus for Protection against Dangerous Fumes and Gases." In criticizing the wisdom of this advice, and the change in first-aid treatment that had followed it, he pointed out that the Registrar-General's Statistical Review of England and Wales for the year 1954 had shown a "sharp rise in deaths from carbon monoxide poisoning", in the two years 1953 and 1954. He suggested that this rise "may possibly have been due to the withdrawal of CO_2/O_2 mixtures, and many preventable deaths may possibly be continuing to occur. The alternative explanation, that there has been a rise in total cases of carbon monoxide poisoning, with proportionate increase in deaths, needs statistics of the annual totals of such cases dealt with by first-aid services in England and Wales during the last seven years."

In view of the obvious importance of this problem I was asked by the Medical Research Council to make a special statistical inquiry.