These changes reverted to normal as spontaneous respiration was re-established.

The method of lung massage suggested is less traumatic and more effective than the direct method suggested by Smolnikoff. When the aetiology of the bronchospasm is unknown or when the spasmolytic drugs are ineffective lung massage by our method may prove to be a life-saving measure.

BIBLIOGRAPHY

- Ariev, M. J., "On the Pathogenesis of the Attack of Bronchial Asthma," *Vrachebnoje Delo*, 1952, 10, 821-886.
- Buyanov, V. M., Riabov, G. A., and Smirenskaya, E. M., "A Case of Lung Massage During Mitral Commissurotomy," *J. thorac. Surg. (Mosk.)*, No. 4, 1959.
- Grinstein, A. M., "The Pathways and Centers of the Nervous System" (monograph), Moscow, 1946.
- Kogan, B. B., "Bronchial Asthma" (monograph), Moscow, 1950.
- Magazanick, Michelson, and Semjonov, "Mechanism of the Bronchial Spasm," *All Union Conference on the Problem of the Pharmacological Reactions Mechanism*, p. 80, Riga, 1957.
- Meyer, G., and Gottlieb, R., "Experimental Pharmacology—the Base of Medical Treatment" (monograph), Leningrad, 1941.
- Mistakopoulos, N. F., "Experimental Treatment of Prostigmine-induced Bronchospasm by Air Massage of the Lungs," *J. thorac. Surg. (Mosk.)*, No. 4, 1959.
- Smolnikoff, V. P., "The Problem of the Bronchospasm," *ibid.*, No. 4, 1959.
- , "Total Bronchospasm and Lung Massage," *J. Anaesthesia*, 1960, 15, 40.
- , "Über die Lungen-Massage," *Anaesthetist*, 8 Band, 12 Heft, December, 1959, p. 350.
- Uspenskaya, E. P., "Clinical and Experimental Study of the New Cholinolytic Drugs Relaxing Bronchial Spasm," *Reports on the IX Conference of the 1st Leningrad Medical College*, p. 110, Leningrad, 1955.
- and Magazanick, Z. G., "Experimental Therapy of Bronchospasm," *Applied Chemistry of Phosphororganic Substances*, p. 356, Moscow, 1957.

REVERSIBLE ACUTE RENAL TUBULAR NECROSIS DUE TO ANTI-Le^a

SUCCESSFUL TREATMENT BY HAEMODIALYSIS

BY

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Since the discovery of the Lewis blood-group system by Mourant in 1946 there have been at least 10 reported instances of transfusion reactions due to anti-Le^a. According to Mollison and Cutbush (1955) these may be mild. We report one case which resulted in acute tubular necrosis of the kidneys, the uraemic and comatose patient recovering after haemodialysis on a Kolff twin-coil artificial kidney. We discuss the serology and emphasize again the dangers of using unmatched blood in transfusions.

Case Report

A married woman aged 20 reported transient intermenstrual vaginal bleeding one week before she was admitted to hospital complaining of pain in the lower

abdomen with vomiting and further minimal vaginal bleeding. Physical examination showed no signs of peritoneal irritation, her blood-pressure and pulse were quite normal, and a diagnosis of threatened abortion was made.

Next day she collapsed quite suddenly with severe abdominal pain; a laparotomy was performed and a ruptured right tubal ectopic pregnancy excised. In this episode her systolic blood-pressure remained at a level of 70 mm. Hg for a period of three hours. She was given 5 pints (2.8 litres) of group O Rh-negative blood which was not cross-matched. After transfusion her systolic blood-pressure rose rapidly to 120 and was maintained. Her general condition was good, and some 400 ml. of urine was removed by catheter. However, on the following day she produced only 180 ml. of urine and on the next day only 60 ml., which was noticeably dark in colour. This degree of oliguria persisted for a further four days. Her only intake was 10% glucose in water by mouth, with 250 mg. of tetracycline q.i.d., on a fluid-balance regime. During the final four days her blood N.P.N. rose from 156 to 238 mg./100 ml. Latterly, persistent vomiting occurred, and she was transferred to our hospital.

On admission seven days post-operatively she was semi-comatose, and was vomiting and screaming irrationally and intermittently. On examination she had facial oedema, generalized twitching, and a blood-pressure of 120/80, and was afebrile; the abdomen showed no special features. The serum ionogram showed serum sodium of 134.5 mEq/l., potassium 6.3 mEq/l., bicarbonate 17.9 mEq/l., and chloride 79.6 mEq/l.; this inferred an abnormally high degree of retention of sulphates, phosphates, or similar anions. Haemoglobin was 12 g./100 ml., and the blood counts showed nothing of note. The blood N.P.N. was 229 mg./100 ml. and creatinine 19 mg./100 ml.; the serum bilirubin was not elevated.

Catheterization provided too small an amount of cloudy yellow acid urine for specific-gravity determination; occasional polymorphs and red cells were present microscopically, and the urine urea concentration was only 800 mg./100 ml.

The clinical impression was of acute renal tubular necrosis, secondary to hypotension at operation or to a haemolytic transfusion reaction. It was felt that the degree of hypotension was possibly not adequate to explain the renal shut-down, and a serological review of blood samples and previous donors was requested.

The patient's coma deepened and she became practically unresponsive to pain stimuli. She was therefore dialysed on a Kolff twin-coil artificial kidney for five hours at an

FIG. 1.—Anti-Le^a transfusion reaction with acute renal failure.

average flow rate of 200 ml. a minute. There was an initial period of hypertension, easily controlled by pentolinium tartrate. At the end of the first hour and a half the puffiness in her eyelids had decreased, and shortly afterwards the hiccups disappeared. Her level of consciousness steadily improved until the end of the run. When awaking she was very confused and agitated, and required amylobarbitone sodium 4 gr. (0.26 g.) per rectum:

Chemical effectiveness of the double-coil artificial kidney is recorded in the form of a graph (see Fig. 1). It produced a precipitous fall in N.P.N. and creatinine followed by a steady rise for three days, but not to the original high levels. Thereafter, these levels returned to normal ranges. They may be regarded as indicators of the degree of retention of sulphates, phosphates, or the unknown toxic products which cause clinical uraemia. The secondary rise is common after a fall due to haemodialysis. It presumably represents the shift of retention products from the extravascular space.

The patient rapidly improved over the next few days and was mentally clear on the day after dialysis. She did not relapse clinically, was ambulatory after two weeks, and was then discharged home to Toronto. The urine volume did not reach a normal level until three days after dialysis, but progressed to a marked polyuria by the sixth day, thereafter dropping gradually to a normal daily volume. From Fig. 2

FIG. 2.—Renal function after dialysis on Kolff twin-coil artificial kidney.

it will be seen that while the urea excretion closely paralleled the urine output the ability of the kidneys to concentrate urea rose much more slowly during the whole period of observation. Before discharge the urine urea reached a concentration of 1.5 g./100 ml. The patient has remained well and free from any signs of renal failure over the ensuing two years. We have no information on her degree of return to full renal-concentration function.

Generally in such cases haemodialysis should be carried out when the following biochemical levels are exceeded: blood urea 300–400 mg./100 ml.,* serum potassium 7 mEq/l., a creatinine level of 15 mg./100 ml., and serum bicarbonate falling below 12 mEq/l. (Kolff, 1955). The clinical supervision of coma was really the factor which decided us to carry out the dialysis.

Serology

The results of blood-grouping tests on the patient were as follows: O MNS P DcE/DcE K— Le(a—) Fy(a+). In the saliva, H substance but no Le^a substance was secreted. The direct Coombs test on the sixth post-transfusion day was weakly positive. Anti-

Le^a was present in the serum ($P=1/1,001$). The titre at 20° C. was 1/4. At 37° C. trypsinized and ficinized Le(a+) cells were haemolysed at a titre of 1/8. The indirect antiglobulin titre was 1/16 on a 48-hour specimen to which complement had not been added. In addition to the effects of the anti-Le^a, some other weak activity could be detected at 4° C., which was attributed to the presence of anti-Le^b.

The donors of the unmatched blood were recalled: four were Le(a—) and only one was Le(a+), with red cells incompatible with the serum of the patient. Since there is no history of transfusions or injections of blood, the anti-Le^a in her serum is presumably of “natural occurrence”; about 5% of Le(a—b—) people possess anti-Le^a in their serum (Mollison, 1959a). Mollison (1959b) pointed out that the complement-binding antibodies such as anti-Le^a bring about quite rapid destruction of incompatible donor cells; if their haemolytic properties are pronounced they may even cause intravascular lysis. Most of the reported cases of reactions due to anti-Le^a have been haemolytic in nature (Mourant, 1946; Krieger and Simmons, 1949; DeVries and Smitskamp, 1951; Brendemoen and Aas, 1952; Lauer *et al.*, 1954; Mollison and Cutbush, 1955; Matson *et al.*, 1955; Peterson and Chisholm, 1958; Merrild-Hansen and Munk-Anderson, 1957; Mouliner, 1959), and the present case emphasizes the danger of potent examples of this antibody.

Discussion

The commonest hazard of unmatched group O blood is the presence in it of incomplete anti-A with haemolytic properties. Although this threat can be largely eliminated by the use of suitable tests in the blood bank, some of the donor antigens will invariably be different from those of the recipient, and may be attacked by the products of previous incompatible transfusions, or pregnancies. When the patient has never been transfused or been pregnant, such incompatible donor antigens may either cause a delayed antibody response that is not immediately harmful or, as in this case, encounter a potent antibody of “natural occurrence.”

Although only about 0.3% of randomly selected people (Kissmeyer-Nielsen *et al.*, 1955) have such a form of anti-Le^a, and only about one in five random donations will be incompatible, nevertheless such a small risk may have decidedly severe effects when it is least expected.

Summary

An instance of uraemic coma due to acute renal tubular necrosis occurring after an anti-Le^a transfusion reaction is reported in a patient without a history of previous transfusions or injections of blood. The anti-Le^a demonstrated in the patient's serum is presumably of “natural occurrence.” Recovery occurred after haemodialysis on a Kolff twin-coil artificial kidney.

The dangers of using unmatched blood for transfusion are again stressed.

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REFERENCES

- Brendemoen, O. J., and Aas, K. (1952). *Acta med. scand.*, **141**, 458.
- DeVries, S. I., and Smitskamp, H. S. (1951). *Brit. med. J.*, **1**, 280.
- Kissmeyer-Nielsen, F., Bastrup-Madsen, K., and Stenderup, A. (1955). *Dan. med. Bull.*, **2**, 202.

*To convert to blood urea nitrogen divide by 2.14.

- Kolff, W. J. (1955). *Med. Clin. N. Amer.*, **39**, 1041.
 Krieger, V. I., and Simmons, R. T. (1949). *Med. J. Aust.*, **1**, 85.
 Lauer, A. von, Clauss, J., and Hoppe, H. H. (1954). *Dtsch. med. Wschr.*, **79**, 1869.
 Matson, G. A., Coe, J., and Swanson, Jane (1955). *Blood*, **10**, 1236.
 Merrild-Hansen, B., and Munk-Anderson, G. (1957). *Vox Sang. (Basel)*, **2**, 109.
 Mollison, P. L. (1959a). *Brit. med. Bull.*, **15**, 92.
 — (1959b). *Brit. med. J.*, **2**, 1035, 1123.
 — and Cutbush, Marie (1955). *Lancet*, **1**, 1290.
 Mouliner, J. (1959). *Bibl. haemat. (Basel)*, **10**, 727.
 Mourant, A. E. (1946). *Nature (Lond.)*, **158**, 237.
 Peterson, E. T., and Chisholm, R. (1958). *Bibl. haemat. (Basel)*, **7**, 59.

SIMPLE TEST-PAPER METHOD FOR THE CLINICAL DETERMINATION OF PLASMA PSEUDACHOLINESTERASE

BY

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Pseudocholinesterase (ChE) is produced in the liver and is normally present in the plasma, though its precise function is unknown. A simple method for the estimation of this enzyme, requiring no elaborate apparatus, would have certain practical advantages. The ChE level of the plasma falls in certain diseases of the liver and has been advocated as a measure of the damage to this organ; it is also of importance in the control of anticholinesterase therapy in myasthenia gravis. It is, however, in connexion with the use of short-acting muscle relaxants such as suxamethonium, which are normally destroyed *in vivo* by ChE, that a knowledge of the enzyme activity of the plasma is particularly desirable. A small proportion of the population, who are otherwise in good health, are deficient in plasma ChE, and if suxamethonium is used on these patients the time of paralysis may be greatly prolonged. This situation may also arise in patients with severe liver damage undergoing general anaesthesia. Preliminary estimation of the plasma ChE in such subjects would reveal this defect.

Methods for the estimation of ChE are based on the amount of acetic acid liberated from acetylcholine either by causing it to release carbon dioxide from a bicarbonate buffer, as in the manometric technique of Ammon (1933), or by observing the fall in pH of a barbitone/phosphate buffered system in a given time (Michel, 1949; Aldridge and Davies, 1952). These methods are accurate but require special apparatus and are laborious and unsuited to clinical work.

More recently, simpler methods based on the colour change of suitable indicators have been published (Caraway, 1956; Rappaport *et al.*, 1959; Smith *et al.*, 1959). Limperos and Ranta (1953) described a method using bromothymol blue in which visual inspection of the mixture of blood and reagents after a short incubation gave an approximate value for the ChE. Herzfeld and Stumpf (1955) described a further simplification of this technique in which filter paper impregnated with a choline ester and bromothymol blue is used. The paper is applied to the plasma or serum

to be tested and the time required for the colour of the indicator to change to that of a standard tint, corresponding to the liberation of a definite amount of acetic acid, is a measure of the ChE activity. These test-papers are now commercially available, and we have compared the results given by them with those obtained by the method of Michel (1949).

Material and Method

The subjects were mostly patients undergoing general anaesthesia. In some patients blood was drawn before and after injection of 2.5 or 5 mg. of the anticholinesterase drug neostigmine methylsulphate; in this way widely differing ChE levels were obtained. Samples from a few patients with jaundice were also included.

The test-papers are yellow strips, 4 by 1 cm., which, when cut in half, provide two pieces 2 cm. long and sufficient for two estimations. Exactly 0.05 ml. of serum or plasma from heparinized blood, free from red cells and haemolysis, is placed at the centre of a perfectly clean microscope slide—that is, one that is free from acid or alkali. The test-paper is applied to the drop of serum so that this is completely absorbed. (N.B. The serum must not be applied to the paper.) The colour of the paper then changes to a dark bluish green. At the same time a stopwatch is started. The test-paper is covered with another clean slide, using gentle pressure and taking care to avoid the inclusion of air bubbles between the slide and paper and to secure even distribution of the fluid. As the action of the ChE proceeds the colour of the paper changes through yellowish green tones to the original yellow. When it matches the colour standard supplied with the test-papers (olive green) the time is noted.

The time between contact of the paper with the serum and matching with the colour standard is inversely proportional to the ChE activity of the serum: thus if the time taken is less than 12 minutes a high plasma ChE level is present, whereas if it is 12 to 20 minutes a normal level exists. Times of 20 to 35 minutes or longer denote a low ChE activity. In practice, observation of the test-paper at 12, 20, and 35 minutes is sufficient to identify the grade of ChE activity.* It is recommended that the strip of standard colour should also be included between two slides. Observations should be carried out in a constant light and at average room temperature (20° C.). Serum or plasma may be kept for a day or two in the refrigerator before estimation and the papers must be protected from moisture, ammonia and the fingers.

Results

There was good agreement between the results obtained with the test-papers and those obtained with Michel's method, except at very low levels (see Chart). The initial ChE values of those subjects who received neostigmine were normal by both methods.

Herzfeld and Stumpf (1955) made a comparison of their method with that of Ammon (1933). They did not claim great accuracy for the filter-paper technique (they were using test-papers prepared in their laboratory) but thought it suitable for routine work: we are in agreement with this. They observed that strongly icteric sera gave results somewhat higher than the true value, presumably because the bilirubin alters the tint of the

*In more recent batches of test-paper these figures have been slightly modified, 7 to 18 minutes being given as normal; the reaction should then be inspected at 5, 18, and 35 minutes.