

Such amplifications of our earlier view had been entertained here prior to the appearance of a communication to the Biochemical Society (April 1948) of Davies and co-workers, in which they refer to a hypothesis of Crane, Davies and Longmuir (to be published), and which "requires that H atom transport by dehydrogenating enzymes is part of the mechanism by which H ions are secreted".

Upon inquiry, it would appear that this hypothesis does not include the supposition that the metabolic hydrogen atoms supply directly the hydrogen ions of the gastric juice in the manner indicated above, and which is an extension of a possible mechanism we had previously put forward<sup>2</sup>. Such would largely simulate the production by the yeast cell of a pH as low as 1.5 in the suspending fluid, and due to free hydrochloric acid.

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<sup>1</sup> Hollander, F., *Gastroenterol.*, 1, 401 (1943).  
<sup>2</sup> Conway, E. J., FitzGerald, O., and Walls, D., *Nature*, 156, 477 (1945).  
<sup>3</sup> Conway, E. J., and Brady, T. G., *Nature*, 159, 137 (1947).  
<sup>4</sup> Davies, R. E., *Biochem. J.*, 40, Proc. xxxv (1946).  
<sup>5</sup> Conway, E. J., and O'Malley, E., *Biochem. J.*, 40, 59 (1946).  
<sup>6</sup> Quastel, J. H., and Wheatley, A. H. M., *Biochem. J.*, 32, 936 (1938).

There is at first sight an evident discrepancy in the distribution of *Ken+* and *Ken-* cells among *A* and *O* groups, and, in a minor way, among *D+* and *D-* cells. The latter does not appear to be significant whereas the former indicates a true difference between the *A* and *O* cell-groups. The  $\chi^2$  and *p* values are given in the following table. Owing to the small number of samples, the Yates adjustment was applied.

	$\chi^2$	<i>p</i>
<i>A-O</i> distribution	4.396	0.036
<i>D+ D-</i> distribution	1.4814	0.224

The observed difference would appear by chance in only 1 in 30 trials for the *A-O* distribution and about 1 in 5 for the *D* distribution. Therefore the *Ken* antigen is found with a significantly higher frequency in the *O* group and is probably related to the *ABO* system. Its genetical transmission is being studied and will be described later.

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### An Antigen Related to the *ABO* System

WE have studied a serum containing an agglutinin, provisionally named 'anti-*Ken*' after the patient's name. This agglutinin was formed by a Polish patient, aged twenty-four, in response to pregnancies: the first ended in a stillborn macerated foetus at term, the second was successfully interrupted by Cæsarean section during the thirty-seventh week. The patient was group *A<sub>2</sub> M CDe CDe Ken-* and her husband *B MN cDE cDE* (or *cDE cde*); we could not distinguish between these genotypes owing to the lack of anti-*d* and anti-*e* sera. The baby proved to be group *O MN CDe cDE Ken+*.

During the second pregnancy, as there were many possibilities of immunization, abnormal anti-*B*, *N<sub>2</sub>c(Hr')*, *d(Hr)* or *E(Rh')* antibodies were searched thoroughly. The characters of the anti-*B* agglutinin remained normal: titre, 1:512, thermal optimum at 4° C., no exaltation by human serum or 30 per cent bovine albumin. At the thirty-second week an agglutinin was found active at 37° C. on certain *O* and *A* cells. It did not correspond to one of the *Rh* antigens mentioned above, nor to the *N* antigen.

Ten days after delivery a suitable amount of serum was obtained. It reacted strongly with the baby's cells and certain *A* and *O* bloods, giving a visual agglutination up to 1:4 and a final titre of 1:32 (in saline) at 37° C. Its activity was markedly increased when 30 per cent bovine albumin was used as diluent.

Testing with *A* and *O* cells of known *D (Rh)* type gave the following results:

Reaction with anti- <i>Ken</i>	+	-	
248 unselected <i>A</i> and <i>O</i> bloods	<i>A</i> cells	46	45
	<i>O</i> cells	102	55
	<i>D+</i> cells	132	83
	<i>D-</i> cells	16	17

### Error in Counting Reticulocytes

WHEN immature mammalian red cells, or reticulocytes, are stained with brilliant Cresyl Blue they may be differentiated from adult red cells and enumerated in a sample as a percentage. If these cells were scattered according to chance, reticulocyte counts would be expected to follow the binomial distribution. In a recent paper, however, Jacobsen, Plum and Rasch<sup>1</sup> have shown that, in their laboratory, reticulocytes appear to be more regularly arranged than this when present in a proportion greater than 10 per cent. They are therefore able to make counts on one sample with remarkably close agreement. Much of the important work on reticulocyte maturation carried out in their laboratory depends on the great accuracy with which this technique is used. It was therefore thought that an independent attempt to confirm this deviation from the binomial distribution would be of interest.

Replicate counts were made on a human case of pernicious anaemia with 13 per cent and on an anaemic rabbit with 20 per cent of reticulocytes. In all, 66,000 red cells were counted. In the human case the counts corresponded fairly well with the binomial distribution; but in the rabbit the counts were less regular.

These results differ markedly from those of Jacobsen, Plum and Rasch, who record increasing accuracy with the higher counts. The discrepancy may have been due to inferior skill both in making the preparations and in counting, for clearly a laboratory worker whose whole time is occupied in counting reticulocytes will attain an unusual ability. On the other hand, this technique gives great scope for unconscious bias. Reticulocytes cannot be distinguished from adult red cells as black from white. The maturation of a red cell is a gradual process, and reticulocytes merge