the normal unadapted level. Still less is there any suggestion that treatment with nitrate has conferred on the cells some intrinsically increased ability to produce nitratase. The increase in total nitratase of the culture of adapted cells is no more than that which occurs with unadapted cells forming the usual amount of " basal " enzyme.

SUMMARY.

Nitratase activities of nitrate-adapted cells of the coliform bacterium " 1433 " growing in a medium without nitrate have been studied.

This preformed nitratase of adapted cells remains unmodified during growth of the rest of the bacterial protoplasm ; so that the enzyme activity per cell gradually falls, during growth, to its normal preadaptive level, by a simple process of " dilution-out."

REFERENCES.

POLLOCK, M. R.—(1946) *Brit. J. exp. Path.*, **27**, 419.
Idem AND KNOX, R.—(1943) *Biochem. J.*, **37**, 476.
Idem AND WAINWRIGHT, S. D.—(1948) *Ibid.*, **29**, 223.
SPIEGELMAN, S.—(1946) *Cold Sprng. Harb. Symp. Quant. Biol.*, **11**, 256.
Idem AND DUNN, R.—(1947) *J. gen. Physiol.*, **31**, 153.
Idem AND REINER, J. M.—(1947) *Ibid.*, **31**, 175.

THE " LEWIS " BLOOD GROUP CHARACTERS OF ERYTHROCYTES AND BODY-FLUIDS.

R. GRUBB AND W. T. J. MORGAN.

*From the Lister Institute of Preventive Medicine, London, S.W.*1

Received April 26, 1949.

A NEW blood group character designated " Lewis " was first described by Mourant (1946) who found that the red blood cells of about 25 per cent of English people were agglutinated by anti-Lewis sera and that the agglutination was independent of the ABO, MN, Rh, P, Lutheran and Kell systems. Jakobowicz, Simmons and Bryce (1947) described a further example of such a serum and Dr. Mourant informs us that he has had several sera of similar character sent to him from the Regional Blood Transfusion Laboratories in this country.

Andresen (1947) reported that he, and independently Dr. Freisleben, had encountered several sera which agglutinated the red cells of 21 per cent of adult Danes. It is now known that the antibody in these sera is identical with the antibody in Mourant's original sera. Andresen (1947) observed that his sera agglutinated the erythrocytes of about 70 per cent of Danish children who were less than 7 months old, and that parents, both of whose red cells failed to agglutinate with these sera could have children whose erythrocytes were agglutinated. He concluded that the agglutinability of the erythrocytes of adults behaves as a recessive Mendelian character, that is, adult persons whose red cells possess this agglutinability are genetically homozygous.

In a further paper Andresen (1948) described an additional antibody which in most of its reactions showed an antithetical relationship to the original Lewis antibody. The red cells of 6 per cent of Group O and about 37 per cent of Group A_1 persons, however, were not agglutinated by either of these sera. Andresen considered that the gene A_1 could have an epistatic action on the agglutinogen defined by this additional antibody.

A revised notation to cover the recent developments in the " Lewis " blood group system has recently been proposed by Andresen, Callender, Fisher, Grubb, Morgan, Mourant, Pickles and Race (1949) and has been used throughout the present paper. The two known Lewis genes recognized by Mourant and by Andresen respectively are now designated *Le*^{a} and *Le*^{b}, the corresponding antibodies as anti-Le^a and anti-Le^b and the gene products as Le^a-substance and Le^b-substance. If the antiserum, anti-Le^a, is used alone in agglutination tests the phenotype of the cells which react is defined as Le(a +), those not agglutinated, as Le(a −).

Our immediate interest in the Lewis blood group system arose from the observation that in a group of 62 persons all those whose red cells were agglutinated by anti-Le^a serum and who were presumably of the genotype Le^{a}Le^a, were nonsecretors of their corresponding A-, B-, H-substances. The results of the complete investigation, a preliminary account of which has been published by Grubb (1948), are now given.

EXPERIMENTAL.

Materials and Methods.

Test sera.

Anti-Le^a sera from nine persons, including the original serum from Mrs. Lewis, were used. Eight of the sera were obtained through the courtesy of Dr. Mourant, who had identified them as anti-Le^a sera. Two of these sera were from persons of Group AB. The anti-Le^a serum " Hughes," which was used throughout the work described in this paper and was kindly supplied by Miss Boorman, Miss Dodd and Mr. Gilbey, of the National Blood Transfusion Service, came from a person with the following group characters: A_2, Le(a −), secretor of A- and H-substances, CDe/CDe, Kell-ve, Lu(a −). This serum is referred to as the " Standard " anti-Le^a serum, and its anti-Le^a character was fully confirmed by us in tests involving a panel of known Le(a +) (9) and Le (a −) (27) bloods. A small amount of a serum " Buckstein " which was considered to be identical with Andresen's second type of anti-Lewis serum, now designated as Le^b antibody, was also available.

Test-cells.

Standard test-cells were Group O cells selected as representing the genotypes Le^{a}Le^a and Le^{a}Le^b. Cells considered to represent the genotype Le^{a}Le^a were strongly agglutinated by the standard serum, were not agglutinated by anti-Le^b and were obtained from a person whose saliva inhibited completely at a dilution of 1:100 the standard dose of anti-Le^a serum. Cells which were not agglutinated by anti-Le^a serum, were agglutinated by anti-Le^b serum and came from a person one parent of whom was of the phenotype Le(a +) were accepted as being heterozygous Le^{a}Le^b cells. The saliva of the heterozygous person showed some power to inhibit anti-Le^a agglutinins.

Agglutination and inhibition tests.

All agglutination and inhibition tests were carried out with once-washed, freshly-prepared erythrocyte suspensions. The degree of agglutination was observed microscopically after smearing a drop of the test material, serum-erythrocyte suspension, on a glass slide. Agglutinations were read after the tests had stood for at least 2 hours at 14–16°. It was observed that after washing the Le(a +) cells six times with saline they were agglutinated by the " standard " anti-Le^a serum to a much lower titre (4) than was normally observed with once-washed cells (16).

The influence of temperature on the degree of agglutination brought about by the " standard " anti-Le^a serum on Group O erythrocytes believed to represent cells of the genotypes Le^aLe^a, Le^aLe^b and of the phenotype Le(a — b —) was observed. The serum failed to agglutinate any of the cells at 37° but reacted weakly with Le^aLe^a cells at 25° and more strongly (titre 16) at 16°. The Le^aLe^b cells were not agglutinated at these temperatures but reacted weakly at 10°. Cells of the phenotype Le(a — b —) remained unagglutinated at 10°.

Owing to the strictly limited amount of the anti-Le^a and anti-Le^b sera available the agglutination technique described by Mourant (1948) for Rh agglutination was employed. The inhibition tests were carried out as follows: The serum (0·01 ml.) was filled into a series of tubes (5 × 50 mm.) by means of a Pasteur pipette graduated at 0·01 ml. and an equal volume of the appropriately-diluted test-fluid was added. The contents were mixed by gentle tapping and allowed to stand at 14–16° for 30 minutes. The erythrocyte suspension (0·01 ml. of a 1–2 per cent red cell suspension) was then added to each tube and the contents mixed. After standing for at least 2 hours at 14–16° the tubes were examined for agglutination.

The tests used to determine the presence or absence of A and B substances in the secretions and body-fluids were performed as described by Morgan and King (1943). The detection of the so-called " O " substance (the H-substance of Morgan and Watkins, 1948) was made by means of an anti-H serum engendered in the rabbit against an artificial antigen built up from purified H-substance and the conjugated protein of the O antigen of *Shigella shigae*. The H activity of the materials was also established by similar inhibition tests which used a serum from the eel *Anguilla vulgaris* (Jonsson, 1944; Grubb, 1949), which serum, at a dilution of 1:100, agglutinated O cells strongly but was without action on A_1B cells.

The pig stomach extracts examined were prepared by autolysis of individual stomach linings at pH 3–4 (Aminoff, Morgan and Watkins, 1946; Bendich, Kabat and Bezer, 1946; Chadwick, Smith, Annison and Morgan, 1949). The human ovarian cyst fluids were from women belonging to Groups A, B, O and AB. Fluids from secretors and nonsecretors within the ABO classification were investigated (Morgan and van Heyningen, 1944).

The preparations of A- and H-substances were obtained by methods already described (Morgan and King, 1943; King and Morgan, 1944; Morgan and Waddell, 1945) and purified until the materials were essentially homogeneous by physical, chemical and immunological tests. Full details for the preparation of these materials will be given elsewhere. The A-substance contained 5·7 per cent N and 9·1 per cent $CO.CH_3$, and showed a *dextro* rotation, $\frac{[\alpha] + 16° \pm 3}{5461}$. The examination of the products of hydrolysis with 0·5 N

HCl revealed that the A-substance contained 37–38 per cent hexosamine (as base) and gave rise to 56 per cent reducing sugars measured as glucose. The α-amino acid N, 2·1 per cent, comprised 38 per cent of the total N. The H-substance contained 5·4 per cent N and was *laevo*-rotatory [α]–30°. The material gave rise to 35 per cent hexosamine (as base) and 53 per cent reducing sugars after hydrolysis at 100° with 0·5 N HCl. A mucoid material, obtained from an ovarian cyst fluid which was devoid of A, B, H, Le^a or Le^b activity was used together with dextran, as a control substance, in inhibition tests. The mucoid contained 5·9 per cent N and showed no significant rotation.

RESULTS.

The examination of 212 erythrocyte specimens from nonrelated adults living in South-East England showed that 47 (22·2 per cent) of them were Le (a +). The salivas of the same persons were tested for the secretion of A-, B- and H-substances and the result showed that 49 (23·1 per cent) were non-secretors of these substances. There was some selection within the ABO classification and too few Group O persons were included.

A rather larger group of similar results representing all specimens examined is set out in Table I, from which it will be seen that of 222 individuals investigated, 57 were Le(a +) and nonsecretors of the A-, B- and H-substances. The remainder (165) were Le(a —) and of these 163 secreted the appropriate A-, B- and H-factors. In this group, therefore, there are two persons who are Le(a —) and nonsecretors of A-, B and H-substances.

TABLE I.—*Correlation Between Le^a Blood Groups and Secretor Character Within the ABO Classification.**

	O.	A_1.	A_2.	A.	B.	AB.	Total.
Erythrocytes Le(a +)							
A,B,H nonsecretors	23	11	6	10	5	2	57
Erythrocytes Le(a —)							
A,B,H secretors	47	45	14	30	18	9	163
Erythrocytes Le(a +)							
A,B,H secretors	0	0	0	0	0	0	0
Erythrocytes Le(a —)							
A,B,H nonsecretors	0	1	1	0	0	0	2
	70	57	21	40	23	11	222
				118			

* This table includes the results recorded earlier (Grubb, 1948).

The secretion of a water-soluble, serologically specific Le^a-substance was investigated by means of inhibition tests made with saliva at three dilutions (1:5, 1:25 and 1:100) using the standard anti-Le^a serum " Hughes," titre 1:16 and Le^aLe^a test-cells. The first 10 saliva specimens examined were investigated with two additional anti-Le^a sera using test cells obtained from four different Le (a +) persons. The only difference observed was that a weaker serum gave a higher inhibition titre, a result which could have been anticipated. A summary of the results obtained with salivas from 80 persons are set out in Table II, from

which it is evident that all persons so far investigated whose red cells are Le (a +) secrete Le^a-substance in the saliva, whereas only some of the persons whose red cells are Le(a −) do so. The salivas of persons from the former group are on the whole more potent than the active salivas obtained from the latter group. A specimen of gastric juice obtained from a person of phenotype Le (a +) contained Le^a-substance.

TABLE II.—*The Results of Inhibition Tests Using Saliva and Anti-Le^a Serum.*

Saliva from persons with	Number of specimens.	Percentage of salivas inhibiting anti-Le^a agglutination. Saliva dilution 1:5	1:25	1:100
Le(a −) red cells	50	84	40	0
Le(a +) red cells	30	100	100	90

The examination of the distribution of the water-soluble Le^a-substance in the body-fluids was extended to a large number of ovarian cyst fluids and the results of inhibition tests, which are set out in Table III, showed that some cyst fluids contained relatively large amounts of Le^a-substance. The cyst fluids were collected earlier for another purpose and it is, unfortunately, not known whether the red cells of the persons concerned were Le(a +) or Le(a −). It will be seen, however, that against a strongly-agglutinating dose of the "standard" serum some cyst fluids could be diluted many thousands of times, and were then able to inhibit completely the agglutinating action of the anti-Le^a serum. One cyst fluid, "UN", showed no significant amounts of A-, B- and H- substances but was rich in Le^a-substance. Preparations of purified and essentially homogenous human Group A-substance and H-substance of both human and animal origin show no significant power to inhibit the agglutination of Group O, Le (a +) erythrocytes by anti-Le^a serum.

TABLE III.—*Showing the Inhibition of Anti-Le^a Serum by Ovarian Cyst Fluids.*

Cyst fluid.	Group.	ABH Secretor (S) or non-secretor (s).	Dilution of cyst fluid inhibiting the agglutination of Le(a +) erythrocytes by anti-Le^a serum. 1:10	1:100	1:1000	1:3000	1:12,000	1:50,000
67	O	S	0	0	0	0	0	0
115	B	S	0	0	0	0	0	0
28	B	s	0	0	0	1	2	2
105	A	S	0	0	0	0	1	2
111	A_1B	S	0	0	1	2	3	3
117	A_1B	S	0	1	2	2	3	3
29	A_1B	s	3	3	3	3	3	3
UN	—	s	0	0	0	0	0	0
Saline	—	—	3	3	3	3	3	3

Degrees of agglutination: O, no agglutination; 1, a few groups of 2–3 cells; 2, larger groups with many free cells; 3, many small clumps.

The results of a few similar tests set up to detect the secretion of a soluble Le^b-substance are given in Table IV. A specimen of mucoid obtained from an

ovarian cyst fluid which showed Le^b activity inhibited a strongly agglutinating dose of anti-Le^b serum at a dilution of $1:2 \times 10^6$. It may be concluded, therefore, that this gene product is also secreted in a water-soluble form.

TABLE IV.—*Showing the Inhibition of Anti-Le^b-Serum by Saliva.*

Donor's red cell phenotype.	Final dilution of saliva inhibiting anti-Le^b serum. 1:3	1:15	1:75
Le(a +)	3	3	3
Le(a +)	3	3	3
Le(a — b +)	0	0	0
Le(a — b +)	0	0	0
Le(a — b —)	3	3	3
Le(a — b —)	3	3	3

Degrees of agglutination as in Table III.

Of 165 persons whose erythrocytes failed to react with anti-Le^a serum, 163 secreted the appropriate A, B, and H factors in a water-soluble form, whereas 2, whose blood belonged to the Groups A_1 and A_2 respectively, failed to do so. The reactions of the blood and saliva specimens from these individuals were confirmed on fresh samples and there appears to be little doubt that these persons are exceptions to the rule that individuals whose red cells are not agglutinated by anti-Le^a serum secrete the appropriate A-, B-, and H-substances.

The erythrocytes of one of the individuals (M.R., Group A_1) were not agglutinated by any of our panel of anti-Le^a or anti-Le^b sera and her saliva failed to inhibit the agglutination of A_2 cells by natural (human) anti-A serum or the haemolysis of sheep cells by rabbit anti-sheep cell serum. The saliva from these two individuals contained neither Le^a- nor Le^b-substance. The red cells of both persons were of the phenotype Le(a — b —).

MISCELLANEOUS OBSERVATIONS.

During the investigation a number of additional observations were made which seem worth recording. For example, no natural anti-Le^a agglutinins were detected in 21 normal rabbit or in 14 normal chicken sera. Specific Le^a-substance was not present in the mucoid materials prepared from 50 different pig stomach linings by acid autolysis and it would appear that preparations of A- and H-substances from this source can, therefore, be used for the neutralization of anti-A and anti-H agglutinins which may be present in human anti-Le^a sera. A mucoid material prepared from sheep submandibular gland (McCrea, unpublished) which showed considerable activity as a receptor substance for heated influenza virus likewise showed no Le^a activity. Anti-Le^a agglutinins were not absorbed by specimens of red cells from six rabbits and were not neutralized by the specific polysaccharide of Pneumococcus, Type XIV, or by the purified O antigen of *Shigella shigae*.

The serum from a person of genotype Le^aLe^a neutralized a strongly agglutinating dose of anti-Le^a serum although at a dilution of 1 : 2 only, whereas the serum from a person of the phenotype Le(a — b —) was without power to inhibit. Le^a-substance is, therefore, most probably present in low concentration in the serum of persons whose red cells are agglutinated by anti-Le^a serum.

The enzymic inactivation of the specific Le^a- and Le^b-substances.

The action of enzyme preparations obtained from culture filtrates of certain strains of *Cl. welchii* (Type B) on purified preparations of the Le^a-substance was examined. It had already been shown that these enzymic preparations inactivate serologically the A-, B- and H-substances (Morgan, 1946. Stack and Morgan, 1949) and that the enzymic activities could to some extent be differentiated by heating the enzyme preparation at 56° for 1 hour whereby the activity against the group substances A and B is lost. The heated solutions, however, are able to destroy the serological character of the H-substance. The Le^a-substance (1·0 ml. of 0·1 per cent solution in buffer at pH 6·4) was mixed with an equal volume of the enzyme solution and incubated at 37° for 2 hours in the presence of toluene. The enzyme solution used was (*a*) unheated, (*b*) heated at 56° for 1 hour, and (*c*) heated at 100° for 15 minutes. After incubation the enzyme-substrate solutions were heated at 100° for 10 min. to inactivate an enzyme present which brings about a sensitization of the red cells used in the agglutination-inhibition test performed subsequently to measure the degree of inactivation of the Le^a-substance. The results of an experiment of this kind are given in Table V from which it will be seen that the Le^a-substance is rapidly destroyed, whereas the enzymic material, after heating for 1 hour at 56° is almost inactive. Similar results were obtained with a partially purified specimen of Le^b-substance.

TABLE V.—*Showing the Inactivation of Le^a-Substance by a* Cl. welchii *(Type B) Enzyme Preparation.*

	Dilution ($\times 10^4$) of Le^a-substance inhibiting the agglutination of O, Le(a +) cells by anti-Le^a serum.								
Treatment of enzyme.	1:2.	1:4.	1:8.	1:16.	1:32.	1:64.	1:128.	1:256.	1:512.
Unheated	0	0	1	2	3	3	3	3	3
Heated 56° 1 hr.	0	0	0	0	0	1	2	3	3
,, 100° 15 min..	0	0	0	0	0	0	1	2	3

Degrees of agglutination as in Table III.

DISCUSSION.

A close correlation of the Lewis blood-group character with the secretor-nonsecretor status within the ABO classification has already been recorded by Grubb (1948) in a preliminary communication involving the data obtained from a relatively small group of adults. The results of the main investigation are communicated in this paper and confirm the earlier finding that persons whose red cells react with anti-Le^a serum are also nonsecretors of the A-, B- and H-substances. More recently, Race, Sanger, Lawler and Bertinshaw (1949) have likewise fully confirmed this interesting relationship. Furthermore, these authors showed that the data obtained from family studies are consistent with Andresen's view that adults whose red cells are agglutinated by anti-Le^a serum are genetically homozygous.

The frequency of bloods in our material giving agglutination with anti-Le^a serum, 22·2 per cent, agrees closely with the figure 22·7 per cent found by

Race *et al.* (1949) in a similar English population. The frequency of nonsecretors of A-, B- and H-substances, 23 per cent, found by us is of the same order as that reported for other populations. The corresponding figures are, for Germany, 22·0 per cent (Schiff), Denmark, 26·0 per cent (Hartmann, 1941) and New York, 18 per cent (Schiff see Wiener, 1946).

Le^a- and Le^b-substances are secreted in a water-soluble form as are the A-, B- and H-substances and, therefore, the terms secretor and nonsecretor should not be used without indicating clearly the group system involved. Le^a- and Le^b-substances resemble the A-, B- and H-substances in general physical and chemical properties. They are heat stable, water-soluble substances of mucoid nature and are readily decomposed by an enzyme in *Cl. welchii* (Type B) culture filtrates. The Le^a-substance occurs in saliva, gastric juice, ovarian cyst fluids and serum. The presence of the Le^b-substance has not been tested for in gastric juice or serum but it has been shown to occur in a water-soluble form in saliva and ovarian cyst fluids.

Le^a-substance was clearly demonstrable in the saliva from 30 persons whose red-cells were of the phenotype Le(a +). The majority of salivas from persons whose erythrocytes were Le(a −) also contained Le^a-substance. These observations are very different from those encountered in the ABO system where, for example, every fifth person belonging to Group A does not secrete A-substance, and A-substance does not occur in the secretions of persons whose red cells fail to react with anti-A serum, that is in those persons who belong to Groups O or B.

It seems as if all persons possessing the *Le*^{a} gene secrete Le^a-substance. One consequence of accepting Andresen's view that persons whose red cells are agglutinated by anti-Le^a serum are of the genotype Le^aLe^a, is that a majority of persons whose erythrocytes fail to react with anti-Le^a serum nevertheless possess an Le^a gene. It is not surprising, therefore, that the specific Le^a-substance may be encountered in the secretions of these persons. In secretions and body-fluids the Le^a-substance is in solution and its presence is demonstrated by means of the very sensitive agglutination-inhibition test. In red cells, on the other hand, the specific Le^a-substance is part of a fixed surface structure, the Le^a agglutinogen, and may be subject to the influence of co-existing heterologous determinant groupings and other unspecific factors which may combine to reduce the sensitivity of the agglutination test. The differences in substrata and technique may, therefore, conceivably make the whole difference between detection (in the saliva) and non-detection (in the erythrocytes) of a single dose of the Le^a-gene. Although it may now be accepted that the Le^a-substance occurs in the secretions of many persons whose red cells seem to lack the corresponding agglutinogen, it can be anticipated that the practical application of this knowledge, the demonstration of the presence of a single dose of the *Le*^{a} gene by means of inhibition tests using saliva, will be beset with difficulties. It is known that within the ABO system the differentiation of secretors from nonsecretors is in certain instances not very satisfactory (Hartmann, 1941) and the results of the present study indicate that the Le^a secretion-nonsecretion phenomenon represents more gradual and continuous variation than is met with in ABH secretion-nonsecretion. It is a matter of conjecture, therefore, whether some of the cases are to be classified as secretors or as nonsecretors of Le^a-substance.

As the great majority of salivas obtained from persons whose red cells fail to react with anti-Le^a serum contain some specific Le^a-substance, only a minority

of salivas could be used for the neutralization of anti-A or anti-B agglutinins in anti-Le^a sera.

In our material all persons who possess Schiff's secretor gene, *S*, have red cells which are not agglutinated by anti-Le^a serum. There is, therefore, a close although inverse relationship between the *Le^a* and *S* genes. It is known from Andresen's results, however, that the majority of these bloods will react with anti-Le^b serum and, furthermore, it is probable that no bloods of the phenotype Le (a + b +) exist. It might be anticipated, therefore, that there is some kind of relationship between *Le^b* and *S* genes. Owing to the very limited amount of anti-Le^{b}serum available we have been forced to confine our tests to a few instances where the erythrocytes or secretions were deemed of special interest. It was thought earlier that a very close relationship might exist between H-substance and Le^b-substance and in consequence a number of specimens of H-substance of human origin were tested for their capacity to neutralize H and Le^b antibody and the two activities expressed as a ratio. The results showed that H-substance and Le^b-substance cannot be identical. Furthermore, we have encountered an individual of the phenotype O, Le(a — b —) who secretes H-substance but not Le^a or Le^b-substances. Dr. Race informs us that he has encountered three persons with similar characters. The frequency of the occurrence of the phenotype Le(a — b —), secretor of A-, B- and H-substances, cannot be estimated on our material as the very limited amount of anti-Le^b serum available has precluded an adequate investigation for the presence of this gene product.

From what is already known of the Lewis system it can be surmised that Le^b-substance and some or all of the A-, B-, H-substances will be frequently found together in the body-fluids and secretions.

It is evident from the results given in Table I and from the findings of Race *et al.* (1949) that the correlation between Le(a +) blood group character and secretor status within the ABO classification, is a very close one. On the basis of probability it would seem impossible that these results have arisen by chance. A simple rule seems to hold and may be expressed as: Adult persons with Le(a —) red cells are nonsecretors of A-, B- and H-substances and adult persons who possess Le(a —) red-cells are secretors of these substances. As a result of this relationship it follows that in adults a good estimate as to the secretor-nonsecretor status of an individual within the ABO classification can be made on the basis of a simple agglutination test with anti-Le^a serum. So far less than 1 per cent of individuals are known to be exceptions.

Individuals whose red cells are agglutinated by anti-Le^a serum most probably possess the Le^a-gene in double dose and they are nonsecretors of the A-, B-, H-substances. Schiff and Sasaki (1932) have shown that persons who are nonsecretors of the A-, B-, H-substances possess the gene *s* in double dose. Does the close correlation of Le(a +) red cell character and non-secretion of the A-, B-, H-substances indicate that the genes Le^a and *s* are identical? We have observed a few individuals of the phenotype Le(a — b —) in whose saliva no A-, B-, H-, Le^a- or Le^b-substances were demonstrable. The absence of these gene products is not due to impaired health or old age and indeed the examination of 10 persons, within the age group 55–80, who were suffering from Addisonian anaemia and were thus nonsecretors of hydrochloric acid, revealed no exceptions to the rule that Le(a —) persons are secretors of the appropriate A-, B-, H-substance. It seems probable, therefore, that the phenotype Le

(a — b —) nonsecretor of A-, B-, H-substances is an accurate reflection of the genetical constitution of these individuals and the existence of such individuals makes it improbable that the genes Le^a and s are identical. Is this an example of linkage in man? If Le^a and s genes are linked they must be very closely linked, otherwise crossing-over would have obliterated the Le(a +)-nonsecretor association in the general population.

If the genes Le^a and s are linked, this linkage is so close that one might place these genes, and consequently the genes Le^b and S, in one genetic system. Furthermore, the general similarity of the physical and chemical properties of the soluble products of the Le^a, Le^b and S genes might be an additional reason for considering that they belong to the same system.

The Lewis system and its relationship to the secretor-nonsecretor phenomenon within the ABO classification cannot be discussed at length in this paper. It is, nevertheless, worth considering briefly. It is suggested that there are three allelomorphic pairs of genes, S and s, Le^a and Le^α, Le^b and Le^β, which occupy contiguous loci. The genes Le^α and Le^β, are allelomorphic to the genes Le^a and Le^b respectively and are unknown at present. The symbols Le^α and Le^β are used here solely to illustrate the hypothesis. Such a system would be similar in many respects to the Rh system and of the eight possible combinations on a single chromosome, four only, $s\ Le^a\ Le^\beta$, $s\ Le^\alpha\ Le^\beta$, $S\ Le^\alpha\ Le^\beta$, $S\ Le^\alpha\ Le^b$, occur at all frequently in our material. The data so far recorded would appear to fit a system of this kind which comprises a closely-packed constellation of three individual genes. We are well aware of the tentative character of the hypothesis. The predictions inherent in the scheme, however, are largely open to experimental proof.

SUMMARY.

1. The examination of blood and saliva of 212 persons has shown that 22·2 per cent are Le(a +) and that these individuals all fail to secrete A-, B- and H-substances. There is, therefore, a very close association between Le (a +) red cell character and inability to secrete in terms of the A, B and H blood-group factors.

2. Le^a- and Le^b-substances are present in secretions of persons of the appropriate genotype and show the same general physical and chemical characters as the A-, B- and H-substances.

3. It seems probable that all persons possessing the Le^a gene secrete Le^a-substance.

4. Some human ovarian cyst fluids are a convenient and potent source of the Le^a- and Le^b-substances.

5. The specific Le^a- and Le^b-substances are inactivated by an enzyme present in *Cl. welchii* (Type B) culture filtrates.

6. Le^b- and H-substances are not identical

7. It is suggested that the Le^a, Le^b, S and s genes belong to one and the same system

8 Among 222 individuals, two nonsecretors of A-, B-, H-substances whose red cells were Le(a —) were found These two individuals were also non-secretors of Le^a- and Le^b-substances and their red cells were Le(b—) A person of the phenotype O, Le(a — b—), who was a secretor of H-substance and a nonsecretor of Le^a- and Le^b-substances was also encountered The occurrence

of persons with these serological characters might be considered to indicate the existence in the Lewis system of additional, unknown genes.

Our thanks are due to Dr. R. R. Race and Dr. Ruth Sanger for much helpful advice during the investigation and to the staff of the Regional Blood Transfusion Depot, Sutton, for a generous supply of bloods and salivas.

REFERENCES.

AMINOFF, D., MORGAN, W. T. J., AND WATKINS, W. M.—(1946) *Nature*, **158**, 879.
ANDRESEN, P. H.—(1947) *Acta. path. microbiol. scand.*, **24**, 616; *Ibid.*, (1948) **25**, 728.
Idem, CALLENDER, S., FISHER, R. A., GRUBB, R., MORGAN, W. T. J., MOURANT, A. E., PICKLES, M. M., RACE, R. R.—(1949) *Nature*, **163**, 581.
BENDICH, A., KABAT, E. A., AND BEZER, A. E.—(1946) *J. exp. Med.*, **83**, 485.
CHADWICK, D. W., SMITH, H., ANNISON, E. F., AND MORGAN, W. T. J.—(1949) *Nature* **164**, 61.
GRUBB, R.—(1948) *Ibid.*, **162**, 933.—(1949) *Acta. path. microbiol. scand.* (in press).
HARTMANN, G.—(1941) 'Group antigens in Human Organs.' Munksgaad, Copenhagen.
JAKOBOWICZ, R., SIMMONS, R. T., AND BRYCE, L. M.—(1947) *Med. J. Aust.*, **2**, 570.
JONSSON, B.—(1944) *Acta. path. microbiol. scand., Supp.*, **54**, 456.
KING, H. K., AND MORGAN, W. T. J.—(1944) *Biochem. J.*, **38**, x.
MORGAN, W. T. J.—(1946) *Nature*, **158**, 759.
Idem AND HEYNINGEN, R. VAN—(1944) *Brit. J. exp. Path.*, **25**, 5.
Idem AND KING, H. K.—(1943) *Biochem. J.*, **37**, 640.
Idem AND WADDELL, M. B. R.—(1945) *Brit. J. exp. Path.*, **26**, 387.
Idem AND WATKINS, W. M.—(1948) *Ibid.*, **29**, 159.
MOURANT, A. E.—(1946) *Nature*, **158**, 237.—(1948) 'The Rh Blood Groups.' London (H.M. Stationery Office.)
RACE, R. R., SANGER, R., LAWLER, S., AND BERTINSHAW, D.—(1949) *Brit. J. exp. Path.*, **30**, 73.
SCHIFF, F., cited from WIENER, A. S.—(1943).
Idem AND SASAKI, H.—(1932) *Z. Immun. Forsch.*, **77**, 129.
STACK, M. V., AND MORGAN, W. T. J.—(1949) *Biochem. J.*, **43**, li.
WIENER, A. S.—(1943) 'Blood Groups and Transfusion.' Baltimore (Thomas).