substance (anti-pernicious anemia factor). Assays of several liver extracts intended for parenteral use in pernicious anemia therapy have shown the presence of small amounts of folic acid, and of larger amounts of the substance(s) formed by autoclaving with acid.

SUMMARY

The occurrence in urine and in acid-autoclaved grass and liver extracts of a substance which appears to participate in the synthesis of folic acid by rat liver *in vitro* is described. A similar effect is produced by synthetic xanthopterin. The effect of these materials might be accomplished by (1) catalysis of the enzymatic synthesis of folic acid; (2) the release of folic acid not liberated by takadiastase from tissue complexes; or (3) their serving as substrate material for the enzymatic synthesis of folic acid. The data presented favor the last hypothesis and suggest that xanthopterin, or a substance derived from it, may constitute a portion of the folic acid molecule. The probable involvement of compounds related to xanthopterin in the formation of hemocytopoietic substances in several animal species is discussed.[20]

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DISTRIBUTION AND HEREDITY OF VARIANTS OF THE RH TYPE[1]

WITH the aid of anti-rhesus immune sera, Landsteiner and Wiener[2] divided human beings into two classes, Rh positive and Rh negative, the former comprising about 84 per cent. of white individuals in New York City. These authors[3] also showed that the Rh factor is inherited as a simple mendelian dominant, independently of the blood groups and M-N types. The Rh factor has proved to be of considerable clinical importance in the etiology of intragroup hemolytic transfusion reactions[4,5] and in the pathogenesis of erythroblastosis fetalis.[6,7,8]

It was early recognized that the Rh factor is antigenically and genetically complex. Thus, Wiener[5] described an anti-Rh serum which agglutinated only about 70 per cent. of all bloods from white individuals, and pointed out that with the aid of this special anti-Rh serum the Rh type can be subdivided into two subtypes analogous to the major subgroups of A. According to the terminology suggested by Wiener and Landsteiner,[9] Rh positive bloods reacting with the special anti-Rh serum (now designated anti-Rh_1) belong to type Rh_1, while the remainder (about one sixth of the Rh positive bloods) belong to type Rh_2. As Wiener and Landsteiner[9] have shown, the types Rh_1, Rh_2 and negative are inherited by means of triple allelic genes Rh_1, Rh_2 and rh, where Rh_1 and Rh_2 are both dominant over rh and and Rh_1 is dominant over Rh_2.

The situation is further complicated by the fact that some bloods of subtype Rh_1 (reacting with anti-Rh_1 serum) do not react with guinea-pig anti-rhesus serum, so that a fourth type is determined in that way.[3] This type (designated as Rh′) is rare, comprising only about 3 per cent. of all white individuals. It should be mentioned that to allow for this special type, it was originally suggested that the standard guinea-pig anti-rhesus (giving about 84 per cent. positive reactions) be designated as anti-Rh_1, and the anti-Rh serum giving 70 per cent. positive reactions as anti-Rh_2.[10] This terminology has been abandoned, however, because it was found that the hypothetical agglutinogens determined in this way do not mendelize like A and B but are apparently "partial antigens" like the factors B_I, B_{II}, B_{III}, . . . of human group B blood, and the factors F_A, A_1, A of group A_1 blood.[11] In the present communication, only the major subtypes, Rh_1 and Rh_2, and not type Rh′, will be included in the discussion.

Recently, Wiener and Sonn[12] have described a serum from a mother of an erythroblastotic infant, which contained a potent anti-Rh agglutinin reacting

20 We are indebted to Miss Helen Ryan for capable assistance; to Dr. W. R. Graham, of the Cerophyl Laboratories, for generous supplies of grass juice powder; to Dr. David Klein, of the Wilson Laboratories, for the powdered liver concentrate (1 : 20). Some of the data in this paper were presented before the Philadelphia Physiological Society on May 18, 1943; an abstract appears in the *Am. Jour. Med. Sci.*, 206: 128, 1943.

1 From the Serological Laboratory of the Office of the Chief Medical Examiner of New York City. Aided by a grant from the Carnegie Foundation and the Committee on Human Heredity of the National Research Council.

2 K. Landsteiner and A. S. Wiener, *Proc. Soc. Exp. Biol. and Med.*, 43: 223, 1940.

3 K. Landsteiner and A. S. Wiener, *Jour. Exp. Med.*, 74: 309, 1941.

4 A. S. Wiener and H. R. Peters, *Ann. Int. Med.*, 13: 2306, 1940.

5 A. S. Wiener, *Arch. Path.*, 32: 227, 1941.

6 P. Levine, E. M. Katzin and L. Burnham, *Jour. Am. Med. Assoc.*, 116: 825, 1941.

7 P. Levine, L. Burnham, E. M. Katzin and P. Vogel, *Am. Jour. Obstet. and Gynec.*, 42: 925, 1941.

8 L. Burnham, *Am. Jour. Obstet. and Gynec.*, 42: 389, 1941.

9 A. S. Wiener and K. Landsteiner, *Proc. Soc. Exp. Biol. and Med.*, 53: 167, 1943.

10 As was first shown by Levine, and confirmed by Wiener, the most common human anti-Rh sera, now designated anti-Rh′, giving about 87 per cent. positive reactions, contain two agglutinins, one corresponding to the 84 per cent. sera (anti-Rh), the other to the 70 per cent. sera (anti-Rh_1).

11 A. S. Wiener, "Blood Groups and Transfusion," 3rd edition, p. 254. Springfield, Ill.: C. C Thomas. 1943.

12 A. S. Wiener and E. B. Sonn, *Jour. Immunol.*, in press.

with only about 30 per cent. of bloods of white individuals. The agglutinogen detected by this serum appears to be of clinical importance in relation to the occurrence of hemolytic reactions in certain Rh positive patients and in many cases of erythroblastosis.[13] Wiener and Sonn[12] have shown that the agglutinogen detected by the new anti-Rh serum is inherited as a simple Mendelian dominant, probably by means of a special allelic gene. Therefore, these observations may also have application in forensic medicine, not only by further increasing the identifiable types of human blood but also by substantially increasing the chances of exclusion in cases of disputed parentage. The purpose of the present communication is to present some new data on the distribution of the major variants of the Rh type, which are of significance in relation to the heredity mechanism.

The Rh reactions of a random series of 280 blood samples from white individuals in New York City are summarized in Table I. It is evident that the

TABLE I

DISTRIBUTION OF THE SUBTYPES OF RH

Type*	Rh_1		Rh_2		Negative		Total
Reaction with special anti-Rh serum	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	
Number	49	141	35	16	1	38	280
Per cent.	17.5	50.3	12.5	5.8	0.3	13.6	100.0
Number in major types	190		51		39		280
Per cent.	67.8		18.3		13.9		100.0

* Determined with the aid of human anti-Rh′ and anti-Rh_1 sera.

new anti-Rh serum makes possible further subdivisions within the major subtypes Rh_1 and Rh_2 and that the incidence of positive reactions is much higher in the latter type. For this and other reasons (see below), it is proposed tentatively to designate the new agglutinin as anti-Rh_2 and the agglutinogen detected by it as Rh_2. Thus, type Rh_1 is now subdivided into type Rh_1Rh_2 (positive with anti-Rh_2 serum) and type Rh_1 proper (negative with anti-Rh_2 serum), while type Rh_2 is subdivided into type Rh_2 proper (positive with anti-Rh_2 serum) and Rh (negative with anti-Rh_2 serum). In the so-called Rh-negative type, there are rare bloods agglutinated by the anti-Rh_2 serum (see Table I). It is obvious that such individuals should now be deleted from the Rh negative type, because the use of their blood for patients actively or passively sensitized to the Rh_2 agglutinogen might prove dangerous.

The hypothesis that the agglutinogen detected by the anti-Rh_2 serum is determined by a gene allelic to *rh* can be tested as follows:

[13] A. S. Wiener, *Amer. Jour. Clin. Path.*, in press.

If we disregard the reactions of the anti-Rh_2 serum, the distribution of the major subtypes in our series is as follows:

$$\text{Type } Rh_1 = 67.8 \text{ per cent.} \quad (1)$$
$$\text{Type } Rh_2 = 18.3 \text{ per cent.} \quad (2)$$
$$\text{Rh negative} = 13.9 \text{ per cent.} \quad (3)$$

and the frequencies of the three corresponding genes are calculated in the usual manner:[14]

$$rh = \sqrt{\text{Neg.}} = \sqrt{0.139} = 37.3 \text{ per cent.} \quad (4)$$
$$Rh_2 = \sqrt{\text{Neg.} + \text{Rh}_2} - \sqrt{\text{Neg.}} = 19.5 \text{ per cent.} \quad (5)$$
$$Rh_1 = 1 - (rh + Rh_2) = 43.2 \text{ per cent.} \quad (6)$$

If, on the other hand, we only take into account the reactions of the anti-Rh_1 and anti-Rh_2 sera and disregard the anti-Rh′ serum, four types are distinguished as follows:

$$\text{Type } Rh_1Rh_2 = 17.5 \text{ per cent.} \quad (7)$$
$$\text{Type } Rh_1 = 50.3 \text{ per cent.} \quad (8)$$
$$\text{Type } Rh_2 = 12.8 \text{ per cent.} \quad (9)$$
$$\text{Negative} = 19.4 \text{ per cent.} \quad (10)$$

The possibility that the agglutinogens detected by the anti-Rh_1 and anti-Rh_2 sera are independent of each other is immediately excluded because $Rh_1 \times Rh_2$ is almost twice as great as $\text{Neg.} \times Rh_1Rh_2$. On the other hand, the hypothesis that the agglutinogens detected by the sera anti-Rh_1 and anti-Rh_2 are determined by corresponding allelic genes Rh_1 and Rh_2 can be tested by calculating the frequencies of the hypothetical genes from the distribution of the four classes above and determining whether the sum of the frequencies equals unity (or 100 per cent.):[15]

$$rh[16] = \sqrt{\text{Neg.}} = \sqrt{0.194} = 44.5 \text{ per cent.} \quad (11)$$
$$Rh_1 = \sqrt{\text{Neg.} + \text{Rh}_1} - \sqrt{\text{Neg.}} = 39.0 \text{ per cent.} \quad (12)$$
$$Rh_2 = \sqrt{\text{Neg.} + \text{Rh}_2} - \sqrt{\text{Neg.}} = 12.0 \text{ per cent.} \quad (13)$$
$$rh + Rh_1 + Rh_2 = 95.5 \text{ per cent.} \quad (14)$$

The agreement between the calculated and expected results is not entirely satisfactory, but this may be due in part to the relatively small size of the series, and perhaps more probably to difficulties with the technique of these delicate tests. The expected frequency of type Rh_1Rh_2 equals $2 \times Rh_1 \times Rh_2$ or 9.4 per cent.; *i.e.*, much less than the observed frequency. This suggests that the problem be further investigated, concentrating particularly on bloods of this type. Of course, additional family studies will also aid in the solution of the problem.

In conclusion, it may be mentioned that the existence of variants of Rh serves to explain the puzzling observations on the differences in specificity of human

[14] *Cf.* A. S. Wiener, "Blood Groups and Transfusion," p. 215, 1943.

[15] *Cf.* A. S. Wiener, "Blood Groups and Transfusion," p. 186, 1943.

[16] This value is greater than that given in (4) because some Rh_2 bloods are now included in the "negative" class. For the same reason, the value given by (13) is less than that given by (5).

anti-Rh sera. Thus, it is now possible to anticipate the behavior of a post-transfusion anti-Rh serum if the Rh subtype of the donor is known, while the quality of the anti-Rh agglutinins in sera from mothers of erythroblastotic babies may be predicted by tests on the blood of the husband or infant.[17] On the other hand, the uniformity in specificity of the guinea-pig antisera is most likely due to the corresponding uniformity of the Rh-like antigens in the red cells of different rhesus monkeys.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE DEMONSTRATION OF THE PROTOZOAN PARASITE OF QUAIL MALARIA BY FLUORESCENCE MICROSCOPY

THE favorable results which have been obtained in development of diagnostic methods for detecting acid-fast bacteria by fluorescence[1] (Hagemann and others) and the results obtained by Bock and Oesterlin[2] in their studies of the action of anti-malarial drugs have suggested the potential value of this method for the diagnosis of malarial infections from blood smears. Subsequent work by the authors has demonstrated that the human parasite, *Plasmodium vivax*,[3] and the organisms of bird malaria, *P. nucleophilum*,[4] and *Haemoproteus* sp.[5] (from California Valley quail) can be stained in a satisfactory manner with fluorescent dyes.

Due to the large size of the parasite and the supply of the *Haemoproteus*, the bulk of the work in these laboratories has been done with this organism.

During the course of investigation positive staining reactions were observed with six fluorochromes. These were applied from saturated aqueous solutions to smears fixed in methyl alcohol as for Giemsa staining. The staining time is from two to five minutes. Alcoholic solutions can be substituted if the smears tend to wash off the slides with the aqueous stain. The six stains and their effectiveness in differentiating *Haemoproteus* are listed in Table 1.

TABLE 1

Stain	Nuclear color	Parasite color	Leucocyte color	Staining intensity
Beberine sulfate	Bright yellow	Golden	Yellow	+++
*Rivanol	Yellow green	Yellow green	Bright yellow	+++
Primulin	Blue	Blue white	Yellow	+++
Coriphosphine 0	Orange	Orange	Bright orange	++
Thioflavin	Yellow	Yellow	Yellow	++
Auramine 0	Yellow	Blue-white	Bright yellow	+

* 2-Ethoxy-6, 9-diamino acridine lactate.

Altering the pH of the staining medium with phosphate buffers showed a very slight increase in staining intensity in the alkaline region.

The apparatus necessary for fluorescence investigations of this nature is relatively simple. The principal innovation in equipment from ordinary microscopy is the use of a G.E. type H-4 high pressure mercury vapor lamp as the light source and a Corning filter No. 5840 which transmits the light between wavelengths of 310 mμ to 394 mμ. Ultraviolet light in this region is invisible but excites fluorescence in the fluorochromes.

The advantages of this technique if developed for the diagnosis of human malaria are many. The staining process is short, simple and reliable; the parasites, if present, stand out brilliantly as brightly fluorescent objects against a dark field; *Haemoproteus* of bird malaria and the *Plasmodia* of human malaria are readily discernible with dry lenses at magnifications not in excess of 200 ×; and the factor of eyestrain is greatly reduced.

Circumstances do not permit the full development of these techniques as applied to malarial diagnosis in this laboratory. All the results thus far obtained indicate that the method offers great possibilities in enhancing both the speed and accuracy of malarial diagnosis from blood smears. It also offers an interesting technique for the study of anti-malarial drugs (many of which are fluorescent) and their action upon the parasites.

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[17] A. S. Wiener, *Amer. Jour. Clin. Path.*, in press.

[1] P. K. H. Hagemann, *Munch. Med. Wschr.*, 85: 1066, 1938.

[2] E. Bock and M. Oesterlin, *Zbl. Bakt.*, 143: 306, 1939.

[3] Furnished by Dr. Martin D. Young and Dr. Mark F. Boyd, U. S. Public Health Service and Rockefeller Foundation, respectively.

[4] Furnished by Dr. R. D. Manwell, Syracuse University.

[5] Furnished by Dr. C. M. Herman, Division of Fish and Game, State of California.

[6] Now with the Tennessee Valley Authority, Wilson Dam, Alabama.

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