HEPATITIS-ASSOCIATED ANTIGEN AND VIRUSES

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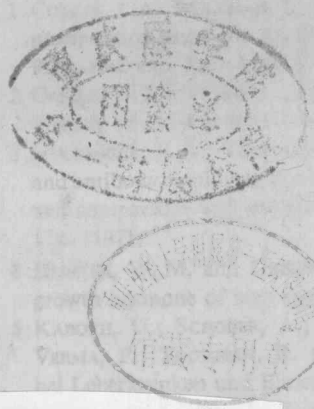

1972

NORTH-HOLLAND PUBLISHING COMPANY — AMSTERDAM · LONDON

Library of Congress Catalog Card Number: 72-86770
ISBN North-Holland-series: 0 7200 7300 4
-volume: 0 7204 7304 7
ISBN American Elsevier: 0 444 10396 1

40 illustrations and graphs, 5 tables

PUBLISHED BY:
NORTH-HOLLAND PUBLISHING COMPANY – AMSTERDAM

SOLE DISTRIBUTORS FOR THE U.S.A. AND CANADA
AMERICAN ELSEVIER PUBLISHING COMPANY, INC.
52 VANDERBILT AVENUE, NEW YORK, N.Y. 10017

PRINTED IN THE NETHERLANDS

Preface

Virus diseases of the liver, which was published in 1970, covered the period of study and research in hepatitis up to 1969. Since then rapid advances followed the discovery of the association between Australia antigen and serum hepatitis or type B viral hepatitis. The interest which Australia antigen has generated in viral hepatitis and the stimulus this accidental discovery has provided for intensive research in the hitherto elusive and frustrating problem seems ample justification for adding another monograph to the vast literature, which has accumulated on the subject since 1969.

This book is an attempt to retell the fascinating story of Australia antigen and it traces the events which followed. The world literature is reviewed, as far as possible, up to March, 1972 and the monograph is presented in the hope that it will stimulate further work and bring additional investigators from different disciplines into this rapidly progressing and challenging field of human medicine.

The work which has been carried out in my own laboratory at the London School of Hygiene and Tropical Medicine is supported by generous grants from the World Health Organization (Dr. W. Chas. Cockburn), the Medical Research Council, Pfizer Ltd., and the Department of Health and Social Security. I would mention in particular the encouraging preliminary results, which have been obtained with the tissue culture of liver cells and organ cultures of human embryo liver. This work, we hope, may possibly provide, in due course, the in vitro model, which is so urgently required for solving the mystery of the biological nature of the serum hepatitis agent. It is hoped that similar techniques may ultimately lead to advances in the field of

infectious or epidemic hepatitis, where virtually no recent progress has been made.

I should like to acknowledge with thanks the work and enthusiasm of members of my staff, Dr. Patricia E. Taylor (now at the Canadian Communicable Disease Centre, Ottawa), Miss Pamela M. Baines, my senior technical assistant, Dr. Susan F. Sullman, Miss Marie A. Bryceson, Mr. A. Bowes, Miss Jill Preece, Mrs. Susan M. Russell, Miss Marilyn Halle, Mrs. Ann Dunne, Miss Deborah Rowe, Dr. M. Monacelli (on study leave from the University of Rome); Mr. N. Byrom, Mr. C. R. Howard and Miss Linda Shaw, who recently joined the Unit and my colleague Dr. R. G. Bird. Much of the work had also been carried out in collaboration with many clinicians and laboratory workers; Dr. Roger Williams, Liver Research Unit, King's College Hospital; Dr. L. J. Farrow, West Middlesex Hospital; Dr. E. J. Holborow, Canadian Red Cross Memorial Hospital, Taplow; Dr. J. Vahrman, Western Hospital; Dr. W. J. Jenkins and Dr. B. Stone, North East Metropolitan Regional Blood Transfusion Centre, Brentwood; Dr. W. D. Brighton, National Institute for Medical Research; Dr. H. E. M. Kay and members of his staff, Royal Marsden Hospital; Dr. R. Vaughan, Dr. J. D. Coombes and Mr. J. M. Leach, Pfizer Ltd., Sandwich, and many others.

Professor David G. Evans, F.R.S. (now Director of the Lister Institute of Preventive Medicine), with his unfailing support, advice and encouragement, is largely responsible for the concept, development and growth of the Hepatitis Research Unit at the London School of Hygiene and Tropical Medicine.

The manuscript was mostly written during long flights to different parts of the world and the text is the result of numerous long and stimulating discussions with many of my colleagues and friends scattered all over the globe.

It is a particular pleasure to thank Miss Irene J. Harris and Miss Karen Saunders, who typed and checked the manuscript with great speed, accuracy and unfailing enthusiasm, over and above their other duties.

Finally, my children Mark and Jane constantly encouraged and urged me to complete the monograph, aided and abetted by my wife Alice and it is to them that this book is dedicated.

Arie J. Zuckerman

Contents

CHAPTER 1

The history of viral hepatitis

The triumvirate rule of the heart, liver and brain probably has its origin in the ancient Egyptian writings. The Egyptians considered the heart as the organ of paramount importance. The Babylonians, on the other hand, regarded the liver as the seat and mirror of the soul and at devination ceremonies the livers of sacrificed animals were examined by the priests (2000 B.C.). Descriptions of disease of the liver and particularly jaundice are to be found in the Babylonian Talmud (5th century B.C.) and jaundice appears to have been common at the time. Hippocrates, during the same period, described epidemic jaundice as the 'fourth kind of jaundice' but the interpretation of the term epidemic is in doubt since yellow bile was regarded as one of the four humours and the agent held responsible for most fevers. It seems that the contagious nature of jaundice was first mentioned in the 8th century A.D. in a letter from Pope Zacharias to St. Boniface, Archbishop of Mainz (Cockayne 1912). Pope Zacharias urged that patients with jaundice should be separated lest others catch the contagion. The first definite description of an epidemic of jaundice amongst civilians was mentioned by Herlitz in Göttingen in 1791. Although Herlitz introduced the term 'icterus epidemicus', Sydenham in London (1624–1689) had already recorded detailed observations of epidemic jaundice. The concept of epidemic jaundice, however, was not accepted.

Virchow (1865) described the pathology of hepatitis after the examination of a single case as a catarrhal obstruction of the common bile duct. The onset of the disease was always associated with a gastrointestinal upset and it was assumed that a microbial infection, not necessarily specific, spread upwards from the intestine to block the bile duct by catarrhal inflammation or cholangitis and thus the term 'catarrhal jaundice' was introduced. Fröhlich

when reviewing 30 outbreaks of jaundice in 1879 reported that there was a suggestion that an infectious process might be implicated in only one outbreak. This fitted well with the accepted view that all varieties of jaundice were essentially obstructive. Eppinger in 1908 taught that all jaundice was obstructive in origin, whether the obstruction occurred in the larger extrahepatic ducts, as in catarrhal jaundice, or in the biliary capillaries, as in cirrhosis. This is surprising since it was widely known that epidemic jaundice occurred during the Middle Ages particularly during wars and jaundice closely followed in importance plague and cholera as the cause of pandemics in Europe.

Yet epidemic jaundice or 'campaign jaundice' was recognized as a military disease much earlier. The excellent historical account of epidemic jaundice by Von Bormann and his associates (1943) refers to an outbreak in Germany in 1629 and they also mentioned an outbreak of jaundice which occurred in the British Army in Flanders in 1743. Monro (1764) carefully documented jaundice in his account of diseases which were common in British troops in Germany from January 1761 to March 1763. Jaundice attacked Napoleon's army in Egypt but it is uncertain whether this outbreak was due to infective hepatitis because of the high mortality rate. During the American Civil War (1861–1865) 71,691 cases of jaundice were reported in the Union Army. Epidemic jaundice occurred during the Franco-Prussian war in 1870 amongst the troops and amongst the civilian population during the siege of Paris. The French referred to infective hepatitis as 'jaunisse des camps' and the Germans as 'Soldatengelbsucht'. During the Boer War in South Africa 5,648 cases of jaundice were recorded and the mortality was low. Large epidemics of jaundice occurred in the Japanese Navy during the war with Russia (1904–1905) and huge epidemics of hepatitis were recorded during World War I particularly in the Middle East theatres. The epidemics of infective hepatitis during World War II attained vast proportions and over 5,000,000 cases occurred in the German armies and civilians alone (Gutzeit 1950), whilst huge epidemics swept through the Allied forces especially in the Mediterranean region (Cullinan 1952). Indeed the number of cases was so large as to influence the strategy of the war. Despite tremendous advances in the knowledge of the epidemiology of hepatitis this infection was again a serious problem in the Israel War of Independence in 1948, the Arab-Israel conflicts in 1956 and 1967, during the Korean campaigns and in South Vietnam.

McDonald in 1908 and again in 1918 first predicted that infective jaundice was probably caused by an agent smaller than a bacterium and postulated

that it was a virus. The viral aetiology of infective hepatitis gained support from then onwards (Stokes et al. 1920; Bergstrand 1930; Findlay and Dunlop 1932; Findlay et al. 1939) and the human volunteer studies finally established the viral aetiology of infective jaundice.

The history of serum hepatitis is much shorter. Lürman (1885) reported the earliest recognized epidemic of serum hepatitis among the shipyard workers in Bremen in 1883. Some cases of smallpox occurred in Bremen and extensive vaccinations were carried out with glycerinated lymph of human origin. One hundred and ninety one of 1,289 vaccinated employees developed jaundice after intervals of several weeks to six months. Several hundred workers employed after the vaccination had been completed and those inoculated with different batches of 'lymph' were not affected. Lürman's paper is a classical example of meticulous epidemiological observations and the translated version by Mrs. Helene Smith is reproduced below with her kind permission and that of Professor J. Garrord Allen of the Department of Surgery, Stanford Medical Centre, Stanford, California.

AN EPIDEMIC OF ICTERUS

BY

DR. LÜRMAN OF BREMEN

(Berliner Klinische Wochenschrift **22**:20–23 Jan. 12, 1885)

Epidemics of icterus have been observed at different times and in various places, and its mention is no rarity in the literature. For this reason, I would not add to these observations, unless the etiology and distribution of the epidemic in question would not be of interest.

Frölich reported on 30 more or less observed icteric epidemics, of which the ones observed in Europe, occurred mainly in army quarters, prisons, i.e., where many people were forced to live together under the same conditions for a long period of time. The etiology of these epidemics is partially obscured, partially believed to be miasmatic, however, mostly they are due to conditions which can produce a gastrointestinal catarrh anywhere. Only in one case, and observed by Frölich himself, he states that the epidemic had the appearance of an infectious disease.

In the following described epidemic, a similar characteristic might be considered.

The epidemic of icterus observed here in Bremen from October 1883 to April 1884, concerns the personnel of 'Weser'-AG. This company (ship and machine building and forge) is located in the west end of the city on the elevated right bank of Weser, and its buildings and shops, etc. reach down to the river. The ground of this development is made entirely of sandy soil. During the observed highest water levels, none of the buildings and terrain had been inundated. In the past two years, no major changes have been carried out on the buildings and similarly there has been no change in the use of the various production materials. No phosphor is being used in the factory.

In the courtyard of the development is located a well, almost on the highest point, which has been in use since 1845, and its water, which is repeatedly chemically tested, is said to have been always good. Only during the summer months, is the water being distributed to the various parts of the factory in five wooden waterbarrels, which after having been cleaned each morning and when necessary also during the day, are filled with fresh water from the well. During the winter, the water supply is taken directly from the well.

The toilets are of the bucket type and are located on six different places of the factory. Sufficient disposal and disinfection with carbolic acid and lime is always carried out.

During the winter of 1883/84, the company employed 1200 to 1500 persons of which only a few had room and board in neighboring quarters, while all the others had their meals in their homes or brought their lunches to work with them in the mornings.

In the previous years, no epidemic of jaundice had been observed among the personnel of this company.

At the end of October 1883, I treated the first few cases of catarrhal jaundice, and their number increased to 33 by the end of November. During the month of December additional 137 cases of jaundice occurred, with 14 more in January 1884, 5 in February and March, and 2 in April. The total reached 191 during the entire period. In reality, however, the number of patients could have been higher, since obviously some persons may not have sought medical treatment. During the period of time mentioned, only occasional cases of catarrhal jaundice were observed in town, which is not unusual for this time of year.

The disease affected the entire personnel of the factory: office workers, technicians, supervisors, laborers, shortly, there were cases of icterus in all social levels. In regard to age, no prevalence was ob-

served. There was the same frequency of icterus observed among the people living in the country as in town. The same was true among the workers of the various shops or the people working in the open air, also in this regard no preference of a certain locality in the factory was noted.

In all diseased, icterus started with signs of abdominal distress, characterized by gastrointestinal catarrh, lasting from 8 days until the appearance of icterus. During that time, patients complained of abdominal pressure and fullness, loss of appetite, nausea and vomiting, dizziness, apathy toward work, etc., mostly there was constipation, rarely diarrhea. With the sudden onset the various subjective symptoms occasionally decreased. In some, however, they increased. In addition itching occurred frequently, and in rare cases seeing yellow; the constipation occasionally was followed by severe diarrhea.

The objective findings did not compare with the severity of subjective symptoms. In all cases there was more or less soreness in the epigastrium, which was very sensitive to touch. Never was there a marked enlargement of the liver. Enlargement of the gallbladder could not be proven, and I was just as much unable to palpate it as a swelling. The whole process was afebrile, and in most cases there was marked reduction in pulse. At the beginning of the icterus, feces became clay-coloured. However, after a few days, they became normal in color and consistency, despite the remaining jaundice or increasing jaundice of the skin and conjunctivae. The urine showed the known icteric coloring in the various nuances without albumen. The Gmelin's test always showed the presence of bile pigment, while the presence of bile acids could not always be proven. The latter test was ordered by the Public Health Department on several cases, to be carried out in an independent chemical laboratory. Subjective, as well as objective symptoms fluctuated, depending on the severity and duration of the illness. Only in one case was there cholemia associated with severe brain symptoms, with insufficient diuresis and subsequent hydrops ascites and anasarca. However, even this severely ill patient recuperated after a six-month sickness. None of the patients had died.

The jaundiced coloring of the skin of the individual patients showed quite variable nuances, being light lemon-coloured in some cases and dark yellow-brown to olive colored in others. Few cases had been noticed in which the well-being of the patient had been hardly affected and the disease passed within 8 to 14 days. Most of the jaundiced

patients suffered for 4 to 6 weeks and again others for more than 6 weeks. The latter showed deep yellow-green color of the skin and conjunctivae, associated with high-grade emaciation and loss of energy. During their illness almost all of the patients lost more or less weight, some of them 12 to 15 pounds. Most of the sick were able to continue with their work, or after a short interruption, to pick it up again, and only the severely afflicted were forced to rest for several weeks.

With regulation of the diet, therapy was a symptomatic one.

As far as the etiology of the described epidemic is concerned, considering both the previous and the following conditions, none of the etiological events leading to an icterus epidemic so far described in the literature fit this picture.

Atmospheric influences have to be excluded, since the epidemic limited itself to the locality of the 'Weser'-AG. company. During the same period of time, there was no case of catarrhal jaundice in two neighboring companies employing an approximate total of 600 persons. There is just as little reason to consider a miasmatic origin of this disease. The high location of the factory provides a strong natural ventilation and there had been no changes in the terrain, undertaken during the year of 1883.

Finally, there is to be mentioned the most important and best known event, the one that can cause gastroduodenal catarrh anywhere, and which despite of its parasitical nature, colds, etc., is due to deficient and wrong diet. However, this too does not fit the picture of this epidemic. As indicated the personnel of this factory come from various social levels, live with a few exceptions, in various parts of the town and country. Therefore, nutrition is entirely dissimilar and variable, and in addition, the families of the married, there was no case of jaundice noticed in the wives or children. The schnaps (brandy) drunk by most of the workers, comes from various sources. As far as the drinking water is concerned, which could be primarily considered a disease carrier, chemical examinations in August and December, described the water to be pure. Also several patients stated they never drank water from the factory well or the water barrels but brought their drinking supply in the form of coffee directly from home.

Since none of the described etiological possibilities was common to all diseased, we have to consider finally a possibility which for statistical reasons, reflecting on the etiological picture of the icterus epidemic, could not be dismissed and that is the revaccination of all

employees and workers of 'Weser'-AG. on August 13, 1883, because of a few cases of smallpox on the premises.

Inoculation took place on the premises in three separate large halls, and was carried out by six doctors. The humanized glycerinized lymph was obtained from a local apothecary, who incidentally, had obtained it from a third source. A name protocol was kept of all inoculated. The doctors used the customary scratch inoculation technique and after each inoculation had been carried out, the instrument was cleaned with a 1% phenol solution by a police officer.

In hall A, 540 persons
In hall B, 466 persons
In hall C, 283 persons

A total of 1289 persons were inoculated. Four metal containers, each containing 100 lymph ampules, were used.

In addition to the inoculated in the halls described, 87 others belonging to the company were inoculated by other doctors and therefore with different lymph, and 50 persons, who had been absent on the day of inoculation, were vaccinated between August 14 and September 1, with the remainder of the left-over lymph.

Of the 540 persons inoculated in hall A, 141 persons became jaundiced.
Of the 466 persons inoculated in hall B, 35 persons became jaundiced.
Of the 283 persons inoculated in hall C, 14 persons became jaundiced.
Of the 50 subsequently inoculated 1 person became jaundiced.
total of 191 persons

Of the 87 persons inoculated by different doctors and with different lymph, none became ill. None of the approximately 500 workers who became employed during the period from the termination of the inoculation to April 1884, became jaundiced.

Since the vaccination was unsuccessful in most, the incidence of the disease was greater in these than those who were successfully vaccinated, and therefore, the result of a successful or unsuccessful vaccination seems to have had no bearing on the development of the epidemic of icterus.

Up to now, there have been no cases reported that would concern workers who were dismissed before the vaccination period. However, there was one case, in which an employee who started to work 14 days before inoculation and was vaccinated. On the other hand, there were 9 cases of jaundiced patients, who in the days or weeks following inoculation left the employment of 'Weser'-AG and found work

somewhere else or were drafted into the army. One laborer, for instance, who had been admitted to the local Diaconissen-Nursing Home because of an injury, was listed there as the only case of jaundice, having developed it shortly after admission. On December 10, resp. December 11, two recruits developed jaundice; they had been admitted to the army in Bremen in October and had been re-vaccinated with workers of 'Weser'-AG on August 13. One previous worker of 'Weser'-AG, who also had been revaccinated on August 13, and who entered the army in Stade, also developed jaundice, while there were no other cases of icterus noted in either the Stade or the Bremer Battalion. It should also be mentioned, that six members of the staff families living on the premises, who had been inoculated, developed icterus and among these were the wife and 10-year-old son of the doorkeeper. They had no contact with either the workers or the workshops.

The revaccination protocol reveals that the jaundice patients had not all been inoculated with the lymph from one and the same metal container.

Except for symptoms associated with a successful vaccination, the patients claimed to have been well during the period of time between the revaccination and the beginning of jaundice.

The inoculation scars revealed nothing worth mentioning.

Considering the facts listed, one cannot possibly avoid calling the mentioned icteric epidemic an infectious disease, the noxious agent of which, by locale and time was sharply circumscribed, that the disease developed into an epidemic with variable incubation periods (fluctuating between 2 and 8 months). The local limits are obvious. The time limit is proven by the fact that it did not occur in workers discharged before August 13, or employed after September 1. Also, among those employed shortly before August 13, or discharged shortly after August 13, jaundice was not observed.

The epidemic itself had a slow start early in October, it reached the highest level in December, thereafter it slowly subsided ending in April.

The etiological question is not really answered on basis of the above data. It has been shown that none of the known etiological phenomena, which can produce an icteric epidemic, is sound enough in our case to account for it, and even the drinking water, a condition of which could have offered the foremost etiological material, cannot be considered on basis of the above data as source for the epidemic. Regarding the latter, it should also be noted that in the administration as well as the

technical office of the company, the rooms contain water carafes, and that one of the six employees in the administration, and two of the six employees in the technical office became jaundiced. Those who became ill, were among the mass-inoculated, while the remaining office workers who did not get ill had been inoculated by their private physicians.

Considering the distribution of cases, in the search for an etiology, one must take into account the revaccination of August 13 as the etiological source of the icterus epidemic. Briefly reviewed, such a causal relation lies in the fact that none of the 87 inoculated on the outside developed the disease, and secondly, of the approximately 500 who were newly employed after the inoculation period, none became jaundiced, and finally the strange situation, in which 9 cases occurred in workers who left this company more or less shortly after revaccination or who were employed by this company before the inoculation period. I am unable to give an explanation for this strange causative nexus'.

The wide-scale introduction and common use of large syringes and long needles with the advent in 1909 of salvarsan therapy in Venereal Disease clinics was soon followed by sudden outbreaks of jaundice. The drug was naturally suspected as a potential hepatotoxin. The Salvarsan Committee of the Medical Research Council, which published two reports in 1919 and in 1922, could come to no decision on the precise cause of jaundice, but toxicity of special batches of the drug was excluded. It is of special interest that in 1917 there was an outbreak of jaundice with 15 deaths at the venereal disease department of Cherryhinton Military Hospital, Cambridge and the MRC committee drew attention to the fact that at the same time there was a small epidemic of jaundice among children in an elementary school near by, affecting 15 children and one adult. Outbreaks of jaundice were later also observed in Diabetic clinics, Tuberculosis clinics and wherever inadequately sterilized syringes and needles, which became contaminated with the blood or serum of a patient or a carrier of the virus of hepatitis, were used.

The term homologous serum jaundice came into use in Britain after the publication of a Ministry of Health memorandum (1943) describing an outbreak of 41 cases of jaundice and 8 deaths which followed the subcutaneous injection of measles convalescent serum into children. However, in 1938 Propert reported that a number of children in an institution developed hepatitis 60 days after the injection of human convalescent measles

serum. Interestingly enough other children, who did not receive serum, developed hepatitis also 60 days later, and it seems likely that this was due to oral transmission as was later established by Krugman and his associates. Earlier in 1937, Findlay and MacCallum drew attention to the occurrence of jaundice following yellow fever immunization and they considered that the jaundice may have been due to some organism injected with the virus or serum. However, they considered that if a hypothetical virus pathogenic for man were directly injected with the inoculum it was surprising that under 3% of persons developed symptoms. Although the presence of a hypothetical virus could not be entirely excluded the evidence against it was very great. Nevertheless, it was concluded that the occurrence of jaundice after yellow fever immunization was analogous to the occurrence of outbreaks of jaundice following antisyphilitic treatment or injections of acriflavine. Similarities were also pointed out between this form of hepatitis and the acute liver necrosis of horses, which was known as 'staggers' in South Africa. In October 1939 about 27% of a group of 304 persons inoculated with one lot of yellow fever vaccine developed jaundice four months after injection. In May 1940 more cases of jaundice appeared in South America in relation to a small number of different lots of vaccine. 1072 people out of 107,000 developed jaundice 12–20 weeks following injection with the incriminated batches of vaccine. However, the largest outbreak of serum hepatitis occurred in 1942 when 28,585 young American soldiers inoculated with yellow fever vaccine developed jaundice and 62 of them died (J. Amer. Med. Ass. 1942). Transmission of the virus of hepatitis by blood transfusion soon became recognized as well as the risk of hepatitis associated with the use of pooled and dried human plasma and human blood products (Zuckerman 1970).

Serological tests for viral hepatitis

Havens (1954) reviewed the early attempts by J.S.H. Gear to devise a specific serological test for the diagnosis of serum hepatitis. A precipitin and complement-fixing antibody was found in the convalescent serum of some patients with hepatitis following immunization with yellow fever vaccine. Gear (1948, 1971) also referred to this precipitin reaction demonstrated in some soldiers who contracted serum hepatitis following immunization against yellow fever in 1942. This antibody reacted with an antigen found in the acute phase serum. Similarly, precipitating and complement-fixing antibodies were demonstrated in convalescent phase sera which reacted with

antigen(s) in acute phase sera and in saline extracts of normal human liver and liver from patients with hepatitis (Sawyer et al. 1944; Eaton et al. 1944). Pollard and Bussell (1953) also described a substance in the acute phase serum of a patient with serum hepatitis which fixed complement with sera from patients convalescent from serum hepatitis, but not with sera from patients recovering from infectious hepatitis or with other forms of jaundice. However, it was not until the discovery of Australia antigen that a specific and reproducible serological test finally became available for the diagnosis of serum hepatitis.

Discovery of Australia antigen.

Ford (1964) defined polymorphism as the occurrence in the same habitat of two or more inherited forms of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation. In polymorphic traits two or more of the genotypes determining variation of the trait are common in the population. Polymorphisms are believed to arise as a result of selective differences between genotypes and they provide convenient systems for the study of inherited discontinuous biochemical variation in man (Blumberg 1964). Included in such systems are the red blood cell antigens (ABO, MNS, P, Rhesus, etc.), sickle cell haemoglobin, haptoglobin, transferrin, glucose-6-phosphate dehydrogenase deficiency, gamma globulin groups and so on. Allison and Blumberg (1961) argued on this basis that patients who are transfused would be likely to receive blood containing proteins which they had not inherited or acquired since donor blood is commonly only typed for the major red blood cell antigens. Some of these differences might, therefore, be antigenic and lead to the development of antibodies in the transfused patients. A systematic investigation of the serum of transfused patients was begun using the simple two dimensional micro-Ouchterlony immunodiffusion technique.

After the examination of some 13 sera from transfused patients in the centre well and a panel of sera from different geographical areas in the peripheral wells, one serum from the transfused patients was found to contain an antibody which reacted with some of the sera in the panel. It was soon demonstrated that this antiserum defined a system of inherited antigenic specificities of the low density β-lipoproteins. This was designated the Ag system (Blumberg et al. 1964) and since then a complex set of specificities has been described. A search for additional such systems was initiated and in 1963 serum from a multiply transfused American haemophiliac was found to