

Transient Alloalbuminaemia Caused By Antibiotic Drugs

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ABSTRACT In the present study we have come across two cases (Paramjit Kaur and Amrik Singh) of transient alloalbuminaemia caused by antibiotic drugs in 1358 pathological sera. The results of our study show that some antibiotics such as neomycin affects the albumin molecule *in-vivo* as well as *in-vitro*, whereas some others like amikacin either show variations *in-vivo* or *in-vitro* like erythromycin and cefran. The effect was seen only in a few individuals and the variations caused were found to be transient in nature.

INTRODUCTION

A number of studies pertaining to drug-albumin interaction resulting in transient types of alloalbuminaemia have appeared in the past. The term "alloalbuminaemia" (from the Greek word 'allo' *i.e.* different) was coined by Blumberg (1969) to describe individuals having variant albumins distinguishable from normal human albumin mainly by charge induced alterations in its electrophoretic mobility (Shrivastava et al., 1972).

The first case of transient alloalbuminaemia of the 'bis' type was reported in a 25 yr old patient suffering from diabetes mellitus. The albumin in this case migrated faster than normal and the anomaly manifested itself only when the patient was under medication, reverting to normal when the disease was under control (Sheulren, 1955). Besides this, reports of alloalbumins appeared in patients suffering from hepatitis (Knedel, 1957) tuberculosis (Nennstiel and Becht, 1957), Wegener's granulomatosis (Earle et al., 1958), acrocyanotic skin changes (Williams and Martin, 1960), Plasmocytoma and lipoid nephrosis (Wieme, 1962) and ascites (Gabl and Huber, 1964) to mention a few.

Taking a cue from the first ever reported case, Arvan et al. (1968) studied the patients receiving intravenous infusions of penicillin or closely related drugs in high doses to check the incidence of transient acquired alloalbumins. Soffiati et al. (1980) also detected a case of transient bisalbuminemia in a patient receiving large doses of penicillin and gentamicin. Galliano et al. (1990) claimed to have observed about 100 transient cases of alloalbuminaemia induced by penicillin therapy or in pancreatic diseases, but the detailed analyses were not carried out to find out the causes of transient alloalbuminaemias.

These studies prompted us to look for the incidence of transient alloalbumins caused by drugs, mainly antibiotics.

MATERIAL AND METHODS

The data were collected from Govt. Rajindra Hospital at Patiala and comprised of 1358 patients receiving large doses of antibiotics (such as neomycin, tetracyclin, amikacin, erythromycin, cefran, gentamicin, septran, penicillin and ampicillin) to study the *in-vivo* effects of drugs (Table 1). A panel of seven normal healthy individuals showing normal albumin A in agarose gels on electrophoresis was tested along with to serve as control for our *in-vitro* experiments.

Fresh samples were obtained from individuals showing altered mobility in the region of albumins approximately two months after recovery when the person was off drugs. Efforts were made to conduct family studies wherever possible to confirm the nature of the anomaly *i.e.* whether transient or inherited.

The albumins were screened by agarose gel electrophoresis using sodium barbital buffer at

pH 8.6 (Berg et al., 1970). The gels were prepared using 1% agarose (International Biotechnologies Inc. No. 3 J07-50) which was free from DNase, RNase and protease. The electrophoresis was allowed to proceed for 90 minutes at 200 volts (constant) in a horizontal system.

Immunoelectrophoretic studies were carried out following the method of Hirschfeld (1960). The rabbit antialbumin anti-serum used was from Sigma Chemical Company, U.S.A. The alloalbumins were designated as fast *i.e.* carrying negative charge when they had mobilities greater than normal albumin A, and slow (positive charge) when the variant albumin band was cathodal to it.

Albumin variants were compared with reference sera: albumins Vancouver (slow +2,

teins.

The drugs were diluted and reconstituted with an equal volume of 0.15M NaCl solution depending upon the concentration of the drug following the method of Arvan et al. (1968). Diluted drug was added to normal sera in the ratio of 3:1. The mixture was incubated at 37°C for one hour prior to electrophoresis.

RESULTS AND DISCUSSION

The results of our studies with different antibiotics tested are given below:

Neomycin: A dark intensely staining band cathodal to the normal albumin band was observed during routine electrophoresis in one case. The patient was a 22 years old female who was operated upon for vaginoplasty and

Table 1: The concentration of the drugs used

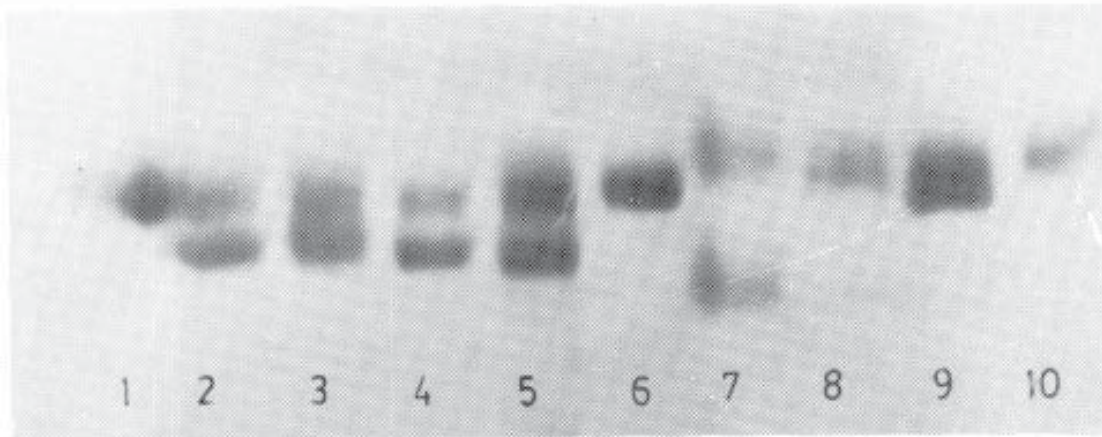
S. No.	Antibiotic used	Source	Concentration
1.	Neomycin	Ranbaxy Laboratories (India) Ltd.	350 mg
2.	Tetracyclin	Sarabhai Chemicals (India) Ltd.	250 mg
3.	Amikacin	Biochem Laboratories (India) Ltd.	250 mg
4.	Erythromycin	Abbott Laboratories (India) Ltd.	250 mg
5.	Cifran	Ranbaxy Laboratories (India) Ltd.	500 mg
6.	Gentamicin	Fulford (India) Ltd.	80 mg
7.	Septran	Burroughs Wellcome (India) Ltd.	400 mg
8.	Pencillin G	Sarabhai Chemicals (India) Ltd.	400, 000 units
9.	Ampicillin	Unichem Laboratories (India) Ltd.	250 mg

501 Glu ----> Lys), Lubeck (Slow +2, 570 Glu ----> Lys), Fortelaza (Slow +2), Mexico (Slow +1, 550 Asp ----> Gly), Cheyenne (slow +1), proalbumins Lille (slow +2, -2 Arg ----> His) and Malmo (Slow +1, -1 Arg ----> Cys), Naskapi (Fast -2, 372 Gly ----> Lys), Navajo (Fast -2) and Cooperstown (Fast-1, 313 Lys ----> Asn) using sodium barbital buffer at pH 8.6 and also using cellulose acetate microzone electrophoresis at pH 8.6 (Courtesy Dr. Frank W. Putnam, Indiana University, U.S.A.), HPLC was also conducted in one case in Dr. Putnam's Laboratory using a Bio-Gel TSK DEAE- 5 PW Column (7.5 x 75 mm) (Bio-Rad) to separate out the normal and variant components of albumin from other serum pro-

was taking neomycin antibiotic post-operatively.

When compared with slow variant albumin controls such as albumin Vancouver (+2), albumin Lubeck (+2), proalbumin Malmo (+2) and proalbumin Lille (+1), it was found to be much more cathodal with +3 mobility (Fig. 1, Lane 7). We sought Dr. F.W. Putnam's opinion regarding this alloalbumin, who suggested that such variations may occur due to proteolytic degradations. However, on immunoelectrophoresis this cathodal band showed immunological identity with normal albumin.

A fresh serum sample collected from the patient after 2 months, showed normal mobility pointing towards transient nature of the al-



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Fig. 1. Comparative mobility of human serum albumin variants in agarose gel electrophoresis at pH 8.6
 Lanes 1, 6 and 10, normal albumin A; lane 2, albumin Samana; lane 3, albumin Vancouver (+2); lane 4, albumin Ropar; lane 5, albumin Lubeck (+2); lane 7, Paramjeet Kaur; lane 8, proalbumin Malmo (+1) and lane 9, proalbumin Lille (+2)

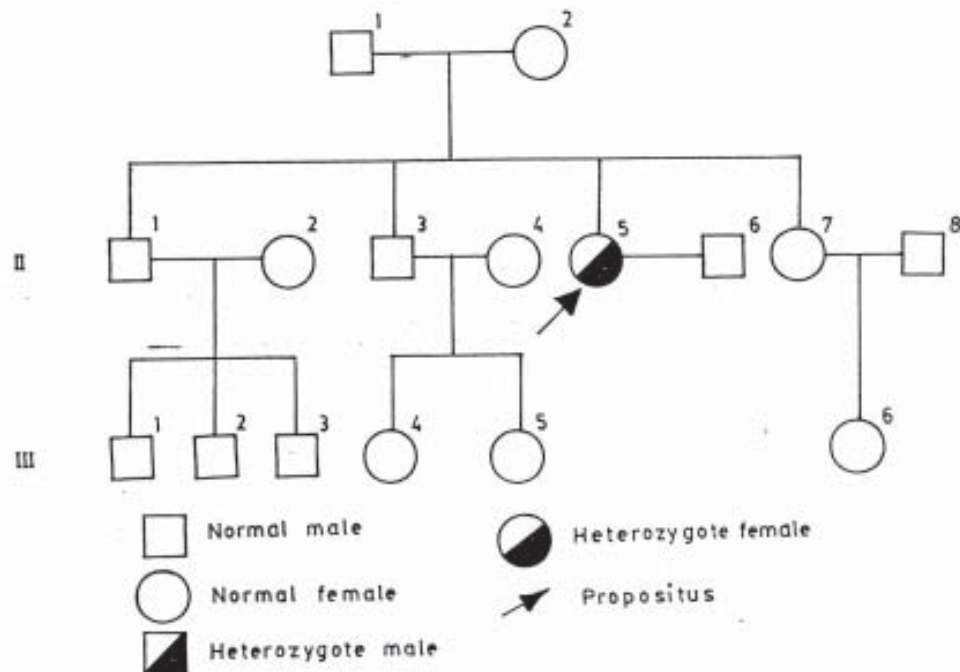


Fig. 2. Transient alloalbuminaemia Pedigree of case -1

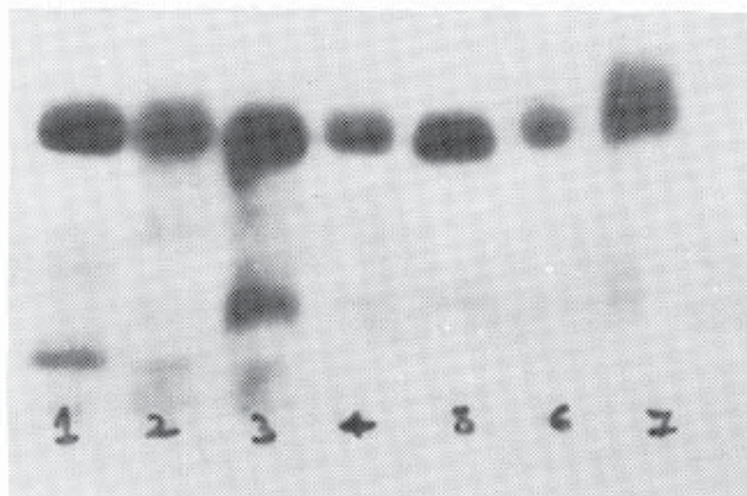


Fig. 3. *In vitro* effect of addition of neomycin to serum albumin

Lanes 1, 2, 3, 5, 6 and 7 normal sera to which neomycin has been added; lane 4, normal serum without neomycin

bumin. The fifteen available members of the family of the propositus investigated had albumin in the normal position further confirming that the trait was transient in nature and perhaps appeared due to the action of the antibiotic taken (Fig. 2).

The *in-vitro* studies in our control series revealed the presence of an additional diffused band only in one out of seven cases studied (Fig. 3, Lane 7) suggesting that different individuals may react differently to different drugs.

Tetracyclin : Fig. 4 (Lane 5) shows results of our studies using tetracyclin *in-vitro*. The slide shows quantitatively low amounts of albumin in the alpha-region, however, a large amount of protein probably bound to drug was observed at the origin.

Amikacin, Cifran and Erythromycin : A fast albumin component was observed in a 49 years old male (Amrik) operated for phylolithotomy. When compared with fast moving alloalbumins Naskapi (-2) and Cooperstown (-1) (Fig. 5, Lane 8) it comigrated with albumin Naskapi showing a mobility of -2. The pa-

tient was prescribed amikacin, cifran, erythromycin and gentamicin as part of his treatment. The sample was sent to Prof. F.W. Putnam who is an authority on albumin studies. He observed the same electrophoretic mobility *i.e.* -2, on comparison with variants albumin Naskapi (-2), Navajo (-2), Cheyenne (+1), Mexico (+1), Fortelaza and Lubeck (+2). Using cellulose acetate electrophoresis at pH 8.6. High pressure liquid chromatography showed two peaks of albumin confirming it as a case of bisalbuminaemia, however, both the peaks were found to be crashed (Fig. 6).

In-vitro studies with Amikacin did not show any variations but with Cifran a case showing slightly faster mobility (-1) was observed. Similar results were observed with erythromycin under similar experimental conditions (Fig. 7) when added to the serum of the same individual whereas the rest of the individuals in our panel did not show any such variation.

Penicillin G : Penicillin G causes variations in the albumin molecule as stated by Arvan et al. (1968) *in-vivo* as well as *in-vitro*. However,

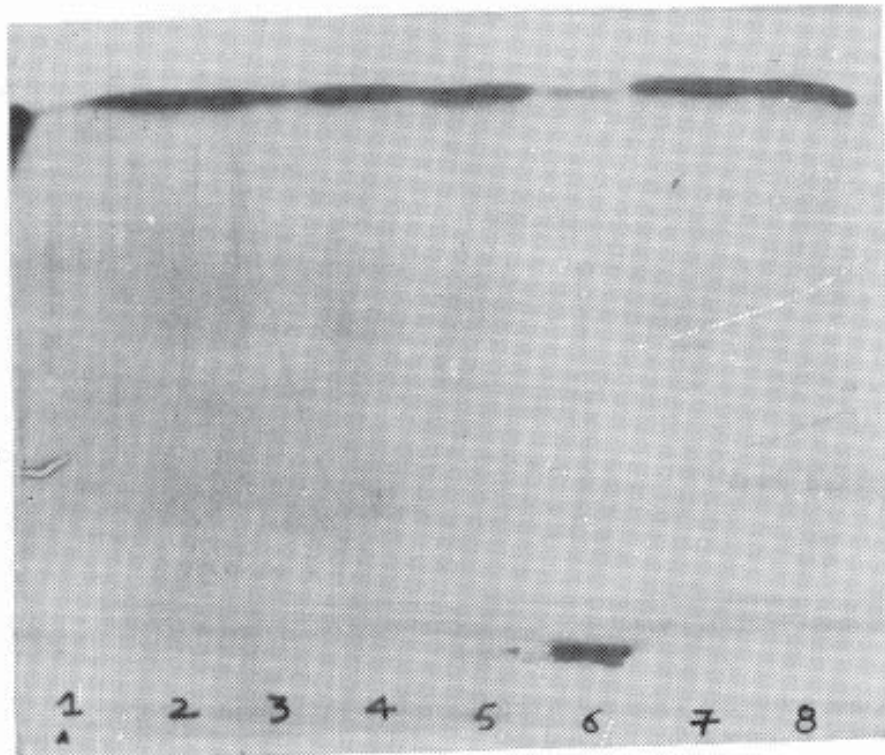


Fig. 4. *In vitro* effect of addition of tetracyclin to serum albumin
Lanes 1, 2, 3, 4, 6 and 7, normal sera without tetracyclin; lane 5, normal serum to which tetracyclin has been added

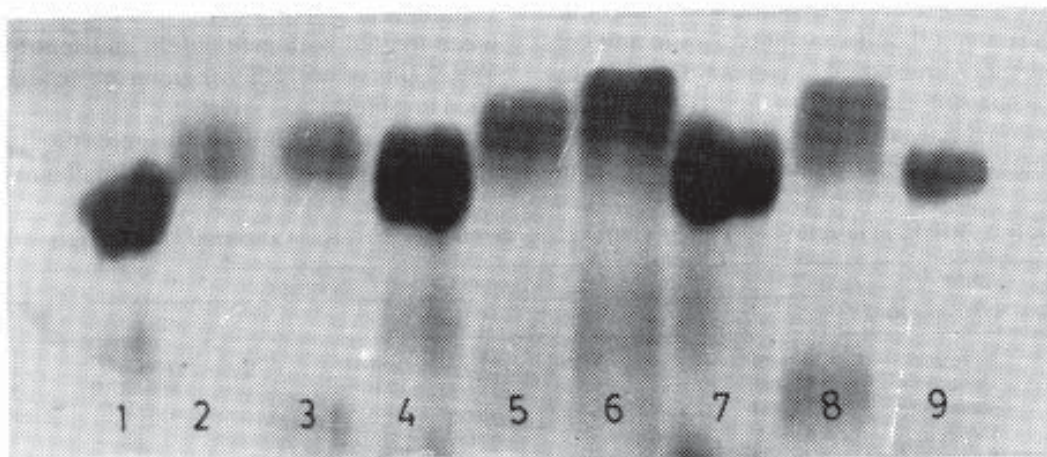


Fig. 5. Comparative mobility of human serum albumin variants in agarose gel electrophoresis at pH 8.6
Lanes 1 and 9, normal albumin A; lane 2, a fast homozygote; lane 3, a fast homozygote; lane 4, albumin Naskapi (-2); lane 5, a fast homozygote; lane 6, a fast homozygote; lane 7, albumin Cooperstown (-1); lane 8, Amrik Singh

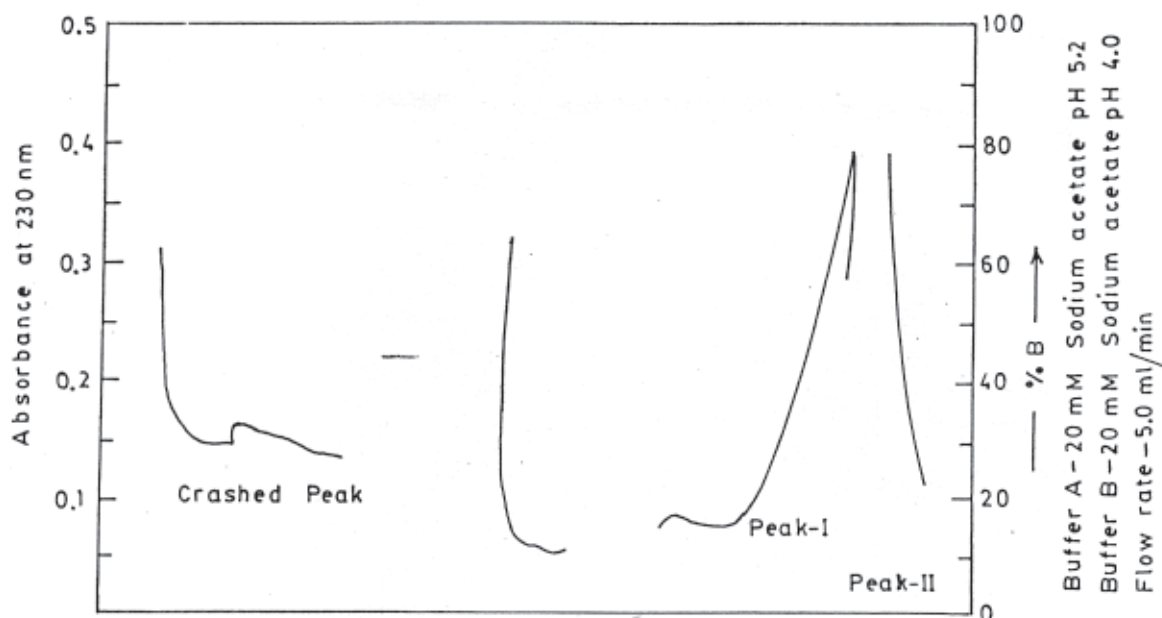


Fig. 6. HPLC elution profile in a Vydac c18 column of CNBr fragments of reduced and carboxymethylated (Amrik)

crease in electrophoretic mobility.

Ampicillin : Though Arvan et al. (1968) observed a fast albumin component with ampicillin, we did not observe any such variations in our study.

Gentamicin and Septran : No apparent variations in albumin were observed *in-vitro* when treated with gentamicin and septran. The results of our control group are summarized in table 2.

Though quite a few studies reporting the apparent presence of transient alloalbumin-

Table 2 : Results of *in-vitro* studies in the control group showing the presence of abnormal albumin component

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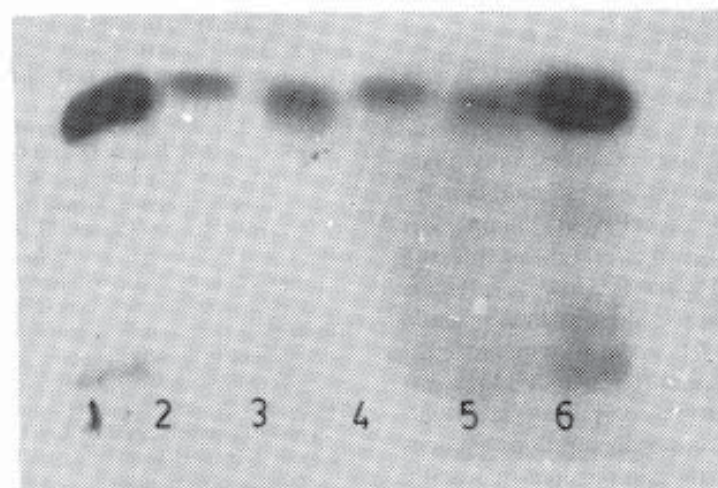


Fig. 7. *In vitro* effect of addition of Cifran and erythromycin to serum albumin
Lanes 1, 6, normal serum; lanes 2, 3 normal sera to which cifran has been added ; lanes 4, 5, normal sera to which erythromycin has been added

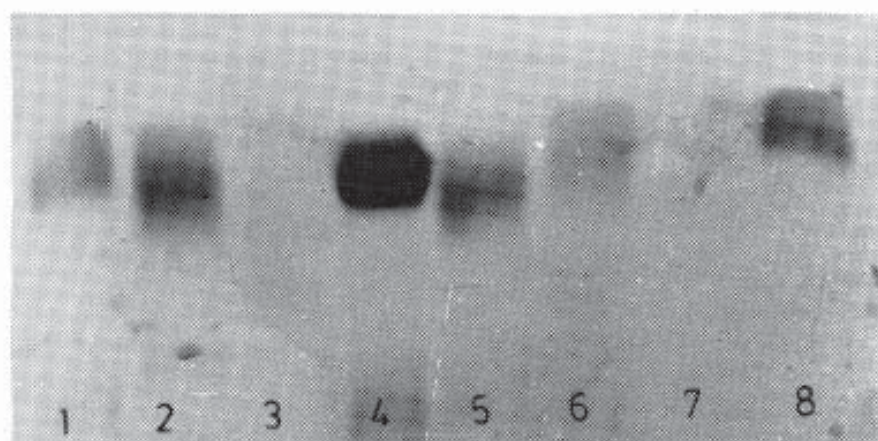


Fig. 8. *In vitro* effect of addition of penicillin G to serum albumin
Lanes 1, 2, 3, 4, 6, 7 and 8, normal sera to which penicillin has been added; lane 5, normal serum without penicillin

aemia are found in literature but many questions still remain unanswered. In general, one cannot accurately predict the albumin binding of a drug from its biochemical nature alone.

From the present study the alteration in the electrophoretic mobility of normal albumin by penicillin and other antibiotics could be confused with some of the inherited albumin variants and may result in oversight of this and similarly charged variants. However, the effect in drug related pseudo-alloalbuminaemia is usually i) dose dependent ii) temporary iii) different drugs affect different individuals differently iv) and the two components are usually present in different concentrations whereas in inherited forms the components are usually equal in amount.

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REFERENCES

Arvan, D.A., Blumberg, B.S. and Melartin, L. : Transient

- "Bisalbuminaemia" induced by drugs. *Clin. Chim. Acta*, **22** : 211-218 (1968).
- Berg, K., Noer, G. and Heilbers, A. : Studies on the genetic polymorphism of the C'3 of human serum. *Clin. Genet.*, **1** : 177 (1970).
- Blumberg, B.S. : The epidemiology of alloalbuminaemia. *Arch. Environ. Health*, **18** : 1-3 (1969).
- Earle, D.P., Hutt, M.P., Schmid, K. and Gitlin, D. : A unique human serum albumin transmitted genetically. *Trans. Ass. Am. Physns.*, **71** : 69 (1958).
- Gabl, F. and Huber, E.G. : In : *Molecular Biology of Human Proteins*. H.E. Schultze and J.F. Heremans (Eds.). Elsevier, New York (1964).
- Hirschfeld, J. : Immunoelectrophoresis-procedure and application to the study of group-specific variations in sera. *Science Tools*, **7** : 18 (1960).
- Knedel, M. : Die-doppel-albuminämie, eine neue erblich protein anomalie. *Blut*, **3** : 129-134 (1957).
- Le-Treut, A., Catheline, M. and Cloarec, L. : Inherited and familial bisalbuminaemia : Acquired transient bisalbuminaemia. *Pathol. Biol.*, **25** : 47-55 (1977).
- Nannstiel, H. J. and Becht, T.H. : Über des erbliche auftreten einer albumin-Spaltung in electrophorese diagramm. *Klin. Wschr.*, **35** : 689 (1957).
- Shrivastava, P.K., Goch, Halina and Zakrzewski, K. : Plurality of human serum albumin. *Biochim. Biophys. Acta*, **271** : 310-319 (1973).
- Scheulren, P.G. : Über Serumeineiss veränderungen beim diabetes mellitus. *Klin. Wschr.*, **33** : 198-205 (1955).
- William, D.J. and Martin, N.H. : Bisalbuminaemia with curious acrocyanotic skin changes (two cases). *Proc. Roy. Soc. Med.*, **53** : 566 (1960).
- Wieme, R. J. : On the occurrence of different types in genetically determined bisalbuminaemia. In : *Protides of the Biological Fluids*. H. Peeters. Proc. 9th Colloq. Bruges Elsevier, Amsterdam (1962).