

esame le altre differenze che appaiono indipendentemente dalle precedenti (e direi nonostante queste) fra varianze e fra medie nei diversi esemplari di *Anas* × *Cairina* e nei vari animali.

Nel complesso le due specie parentali si sono rivelate nettamente differenti per tenore medio in acido desossiribonucleico (è maggiore di circa il 20% in *Anas platyrhynchos*).

Negli ibridi la variabilità dei singoli dati è stata più elevata, ma solo negli esemplari di sesso maschile, di quella nelle specie parentali; la tendenza alla bimodalità osservabile negli istogrammi può indicare la possibile presenza di due popolazioni di valori in ogni individuo.

Il tenore medio dell'ibrido *Cairina* × *Anas*, per cui disponevo solo di due esemplari maschi, è stato corrispondente a quello della specie paterna, che presentava i valori più bassi.

L'ibrido reciproco ha mantenuto nel complesso un valore intermedio fra quelli delle specie parentali; però con una notevole differenza fra gli esemplari dei due sessi: mentre quello di sesso maschile ha presentato un valore medio uguale a quello dell'ibrido reciproco, nonché a quello della specie materna (con i valori minori), i due esemplari di sesso femminile hanno fornito un valore corrispondente a quello della specie paterna (con i valori maggiori).

È suggestiva la corrispondenza fra queste osservazioni (medie di valori e loro distribuzione) e la constatazione della profonda sterilità dei maschi di entrambi gli ibridi, mentre le femmine studiate erano giunte a deporre uova, indicando un maggior equilibrio dei loro gameti, confermato da una più regolare distribuzione dell'acido desossiribonucleico per nucleo.

Questi dati si prestano ad un confronto interessante con quelli già pubblicati riguardanti l'asino, il cavallo ed il mulo<sup>1</sup>: quest'ultimo presentava, indipendentemente dal sesso, un tenore intermedio fra quelli delle specie parentali; non erano presenti inoltre le irregolarità nella distribuzione dei singoli dati, riscontrate in questi ibridi di anitra, particolarmente nei maschi.

Si tratta di risultati che invitano ad una estrema prudenza, già in precedenza espressa<sup>1</sup>, prima di giungere a conclusioni generali: ulteriori chiarimenti sono da attendersi da un'estensione delle ricerche ad altri ibridi, prendendo in esame anche le cellule germinali oltre che le somatiche e, nel caso di fertilità, anche solo relativa, pure i prodotti della seconda generazione e dei reincroci ottenibili.

Per quanto compiute in campo diverso meritano un cenno, perchè si allacciano a tali questioni, le osservazioni di LEUCHTENBERGER e coll.<sup>6</sup> che in spermatozoi umani notarono caratteristiche alterazioni della distribuzione e del contenuto medio in acido desossiribonucleico, in rapporto ad alterazioni delle capacità riproduttive.

È di un certo interesse infine confrontare questi dati istofotometrici con i caratteri cromosomici; in MATTHEY<sup>7</sup> ed in WHITE<sup>8</sup> sono raccolte e valutate criticamente diverse osservazioni sulle specie qui esaminate ed i loro ibridi. Si nota come non vi sia accordo completo sul numero complessivo dei cromosomi e sui caratteri degli eterocromosomi: comunque le due specie parentali differirebbero di pochissimo e così pure l'ibrido. Al con-

trario il contenuto in acido desossiribonucleico si presenta notevolmente diverso, ciò che indicherebbe una indipendenza di questo valore dal numero dei cromosomi. Non è possibile per ora accertare la ragione di questa diversa carica in acido desossiribonucleico per cromosoma, in specie con cromosomi morfologicamente così simili.

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### Summary

The desoxyribonucleic acid content in nuclei of erythrocytes in *Anas platyrhynchos*, *Cairina moschata* and their direct and reciprocal hybrids is studied by the quantitative method. A significant difference was found between the amounts in the two parental species. The hybrids reveal an irregularity in behaviour of their means and distribution of contents. This seems to be dependent on their origin and sex. These findings are discussed and critically compared with other data from the literature, concerning the desoxyribonucleic acid content and chromosome-characteristics.

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### Relationship Between Passive Hemagglutination of Tanned Red Cells Coated with Fraction I and Fibrinolysis in Cirrhosis of the Liver

The BOYDEN method<sup>1</sup> has been applied by KISSMEYER-NIELSEN<sup>2</sup> to the fixation of platelet extract on the surface of red cells and to the study of rabbit antibodies against human thrombocytes. SAUER and VAN LOGHEM<sup>3</sup>, KISSMEYER-NIELSEN<sup>4</sup>, DAUSSET, BERGEROT-BLONDEL and BREC<sup>5</sup> used this method for the study of hemorrhagic syndromes in human subjects. It has been shown by Ducos<sup>6</sup> and confirmed by us, that the platelet extract can be replaced by fibrinogen.

It appears to us that this reaction was frequently strong in severe cases of cirrhosis of the liver associated or not with a hemorrhagic syndrome but always accompanied by moderate thrombocytopenia. In studying this reaction, we noted that it was: (1) negative when the serum was obtained immediately after clotting by the use of mechanical agitation with glass beads, and (2) strongly positive when the serum was separated from the clot after an incubation period of 1 to 2 h at 37°C.

These facts led to the investigation of the role of the clot in this reaction. We made a cross experiment of clots the results of which are given in the Table I. As can be seen, the reaction is positive only, when the patient's clot was incubated in pathological as well as in normal sera, or even in physiological saline. *During the incubation the patient's clot liberates a substance capable to react with*

<sup>1</sup> S. V. BOYDEN, J. exper. Med. 93, 107 (1951).

<sup>2</sup> F. KISSMEYER-NIELSEN, Vox Sanguinis 3, 123 (1953).

<sup>3</sup> A. J. SAUER and J. J. VAN LOGHEM, Vox Sanguinis 4, 120 (1954).

<sup>4</sup> F. KISSMEYER-NIELSEN, Sang 26, 117, 123 (1955).

<sup>5</sup> J. DAUSSET, Y. BERGEROT-BLONDEL, and H. BREC, Sang 9, 861 (1955).

<sup>6</sup> J. Ducos, National Congress of Blood Transfusion, Bordeaux (1956).

<sup>6</sup> C. LEUCHTENBERGER, F. SCHRADER, D. R. WEIR e D. P. GENTILE, Chromosoma 6, 61 (1953).

<sup>7</sup> R. MATTHEY, Les Chromosomes des Vertébrés (Librairie de l'Université, Lausanne 1949).

<sup>8</sup> M. J. D. WHITE, Animal Cytology and Evolution (University Press, Cambridge 1954).

## Passive Hemagglutination

*Mixture of a pathological blood (cirrhosis of the liver) with a normal blood:*

Pathological serum\* incubated during 2 h at 37°C in contact with:

|                                                                   |                   |
|-------------------------------------------------------------------|-------------------|
| Pathological red cells . . . . .                                  | —                 |
| Normal red cells . . . . .                                        | —                 |
| Pathological platelets . . . . .                                  | —                 |
| Normal platelets . . . . .                                        | —                 |
| Clot of the pathological plasma (without anticoagulant) . . . . . | + + $\frac{1}{4}$ |
| Clot of the normal plasma (without anticoagulant) . . . . .       | —                 |
| Without any contact . . . . .                                     | —                 |

Physiological saline incubated during 2 h at 37°C in contact with:

|                             |                    |
|-----------------------------|--------------------|
| Pathological clot . . . . . | + + $\frac{1}{16}$ |
|-----------------------------|--------------------|

Normal serum\* incubated during 2 h at 37°C in contact with:

|                                                                   |                   |
|-------------------------------------------------------------------|-------------------|
| Normal red cells . . . . .                                        | —                 |
| Pathological red cells . . . . .                                  | —                 |
| Normal platelets . . . . .                                        | —                 |
| Pathological platelets . . . . .                                  | —                 |
| Clot of the normal plasma (without anticoagulant) . . . . .       | —                 |
| Clot of the pathological plasma (without anticoagulant) . . . . . | + + $\frac{1}{2}$ |
| Without any contact . . . . .                                     | —                 |

Physiological saline incubated during 2 h at 37°C in contact with:

|                       |   |
|-----------------------|---|
| Normal clot . . . . . | — |
|-----------------------|---|

\* Sera immediately separated after clotting by the use of mechanical agitation with glass beads, incubated at 37°C with and without contact, and then heated, at 56°C for 30 min. All reactions are negative without heating at 56°C.

*fraction I coating the tanned red cells.* This substance agglutinates these red cells only when it has been heated previously for 30 min at 56°C.

The immunological nature of this substance is doubtful. Experiments have shown at least two different kinds of behavior in relation to rabbit antibodies against human platelets: (1) the rabbit antibody is formed in the serum and is not liberated by the incubated clot; (2) it does not require heating at 56°C to promote hemagglutination.

The relation between hemagglutination and fibrinolysis has been demonstrated by two different approaches:

(a) Clots of patients, in which the reaction was found strongly positive, have marked tendency to fibrinolysis. This was seen after incubation of blood, diluted with equal volume of physiological saline, at pH 7.2, under sterile conditions, during 24 h at 37°C. In all cases complete lysis has been observed.

(b) 1 cm<sup>3</sup> of normal blood, clotted at 37°C in the presence of 15 mg of purified bovine fibrinolysin, gives off, after 2 h of incubation at 37°C, a serum capable also to promote the reaction. Complete lysis of the clot is observed after 24 h under the same conditions.

**Conclusions.** In the course of fibrinolysis of the clot, which is an especially rapid process in liver cirrhosis, this clot liberates a substance which is capable, after heating of 30 min at 56°C, to react with tanned erythrocytes coated with a plasmatic substance found in fraction I. It is probable that this phenomenon is not limited to liver cirrhosis, but can be found also in other syndromes associated with fibrinolysis. It is believed this is an exacerbation of a normal physiological process.

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*National Centre of Blood Transfusion, Paris, March 15, 1956.*

## Résumé

Au cours de la fibrinolyse, particulièrement précoce au cours des cirrhoses du foie, le caillot libère une substance capable après chauffage de 30 min à 56°C de réagir avec

des hématies recouvertes d'une substance plasmatique présente dans la fraction I.

Il est probable que le phénomène n'est pas limité aux seules cirrhoses hépatiques, mais s'étend à d'autres syndromes hémorragiques (fibrinolytiques) et qu'il s'agirait de l'exagération d'un processus physiologique normal.

### A Dialyzable Factor from Plasma Responsible for the "Viscous Metamorphosis" of the Blood Platelets. Its Role in Clot Retraction and Haemostasis\*

During coagulation of normal blood, the thrombocytes undergo a series of morphological changes which lead finally to their total disintegration. This phenomenon is generally called "viscous metamorphosis" of the platelets, and its most easily observed manifestation consists in agglutination and clumping of these cells. Since it is known that in most cases thrombus formation in venous thrombosis starts out from agglutinated and fused platelets adhering to the wall of a blood vessel, attention in recent years has been focused on the factors involved in the thrombocyte agglutination<sup>1</sup>. A new dialyzable factor was discovered in the course of the following experiments.

Some authors still believe *clumping* of the thrombocytes to be a secondary phenomenon due to the conversion of small amounts of fibrinogen to fibrin or pro-fibrin<sup>2</sup>, in spite of the fact that platelets in afibrinogenemic plasma agglutinate normally<sup>3</sup>, and that elec-

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<sup>1</sup> Throughout this paper agglutination is understood in the sense of describing the first manifestation of viscous metamorphosis, thus excluding nonspecific aggregation or serological reaction of intact cells with antiplatelet antibodies.

<sup>2</sup> K. APITZ, *Erg. inn. Med.* 61, 54 (1942). — K. LENGGENHAGER, *Weitere Fortschritte in der Blutgerinnungslehre* (Georg Thieme, Stuttgart, 1949), p. 7 and 177.

<sup>3</sup> B. ALEXANDER, R. GOLDSTEIN, L. RICH, A. G. LE BOLLOCH, L. K. DIAMOND, and W. BORGES, *Blood* 9, 843 (1954).