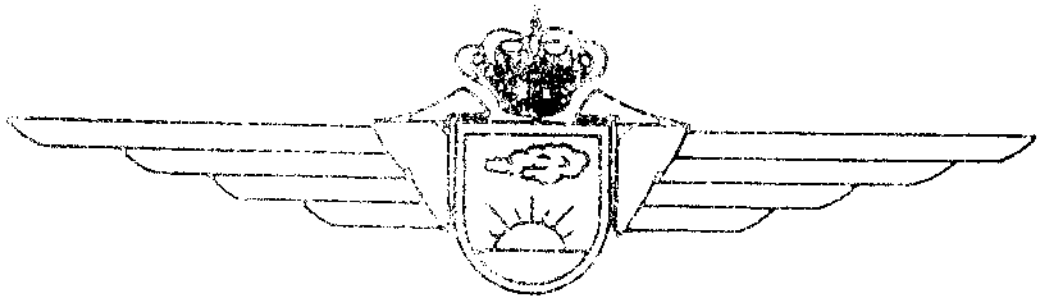
BELGIAN AIR FORCE

METEOROLOGICAL WING



STRATEGIES FOR THE IDENTIFICATION OF BIRD REMAINS FROM BIRDSTRIKES

SURVEY AND ADVANCED APPROACH BY BIOCHEMICAL ANALYSIS OF TISSUES

IN COLLABORATION
BY

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1. Introduction

In 1965 the Belgian Air Force recorded 100 birdstrikes (BS), among these 44 with damage.

Actions to avoid or diminish birdstrikes are not only based on aerodrome measures and birdtam informations but also on information about the bird species involved, their behaviour and their way of living. This kind of information may be useful, e.g. to render airfields and their surrounding less attractive to some bird species.

Different bird species may respond in a different way to the same dispersal methods. It is therefore important to be able to identify them up to the species level in order to take the exact preventive measures.

Since several years, a group of biologists attached to the Meteorological Wing of the Belgian Air Force and a research laboratory at the University of LEUVEN collaborate at the same question within the frame of B.S.C.B.. This collaboration is of interest for both parties since a lot of scientific information concerning the flying habits of birds can be obtained from these birdstrikes.

This paper deals with some recent developments in the identification of bird species by means of biochemical techniques.

2. Species identification

2.1. Bird strike report

BS of the Belgian Air Force are reported by the pilot and (or) by the maintenance service personel. A special form (ICAO based) is filled out and sent to the Zoological Institute of the K.U.L. together with the bird remains in order to get the data necessary for an exact identification of the bird. In this way, Air Force and scientists get the maximum out of the available data.

2.2. Feather examination

2.2.1. Macroscopic :

Direct examination of the size, shape, colour and pattern of the feathers is the most at hand method to identify the bird : this is carried out at the Zoological Institute (K.U.L.). The weight of the remains is only a supplementary value since when large parts of the bird are recovered, the plumage is mostly amply sufficient for a correct identification of the bird. This method is simple, cheap and may lead to rapid results. In 1984 and 1985 approximately 50 % of the remains could be identified up to the species level.

2.2.2. Microscopic :

This method is based on the work of T.C. Brom of the University of Amsterdam. The feathers are mounted under a coverslip and examined under the microscope. The downy parts at the base of the shaft of the feathers particularly possess a microscopic structure characteristic for each family and even species of bird. These plumes are very suitable for microscopic examination because most of the times they can be found sticking in blood on the aircraft. It is very difficult to identify them macroscopically (14th meeting B.S.C.E. THE HAGUE).
The results of previous investigations show that several samples of bird remains could be satisfactorily identified, even when burned and carbonized by the engines (Veilig Vliegen Jan 80).

This technique was a great progress in the positive identification of birds so that the number of unknowns decreased.
A study about the effect of succesful identifications on BS statistics of the RNAF by L.S. Buurman and T.C. Brom stipulates that the identified samples increased with the factor 10 from 5 % in 1974 to 52 % in 1976.
Attention was also drawn on the fact that the relative importance of a certain bird species is dependent on the identification ratio and the ratio of BS with damage. Birds that are easy to recognize, such as white and large ones, also influence the statistics in their favour. The same phenomenon occurs for BS with sufficiently intact remains, e.g. during take off or landing. Otherwise, the statistics may underestimate some species and overestimate another. This might lead to the wrong conclusions as to the preventive measures to be taken and as to an optimal use of the available means.

2.3. An advanced approach : Biochemical analysis of tissues

Although the feather remains allow identification in a great number of cases, still to much remain unsolved, in casu those where no suitable feathers are available. However, most of the times, some blood or tissue can be recuperated.
Several biochemical techniques have been suggested to analyse these samples. The biological molecules of importance are proteins. Each species has its own typical set of proteins.
Principally, these techniques are all based on the same idea : the main components in the tissues are separated and visualized. This always occurs in a specific pattern for a specific species ; in this way one can get as it were a kind of fingerprint of the species involved. The obtained pattern is compared to those stored in a kind of library that contains all possible patterns of birds to be expected in the area.
The most important techniques are shortly discussed with their main advantages and drawbacks.

2.3.1. Thin layer or paperchromatography

A small sample of tissue or blood is dissolved in a "running buffer" (mobile phase). A small spot of this solution is positioned on a special kind of filtration paper (stationary phase)(fig 1A). When the paper is brought into contact with the running buffer, the solution is sucked up (fig 1B). In this way, the proteins are transported and separated, depending on their molecular weight and other chemical properties. This results in a typical band pattern that can be stained and which serves as a kind of fingerprint for the proteins involved.

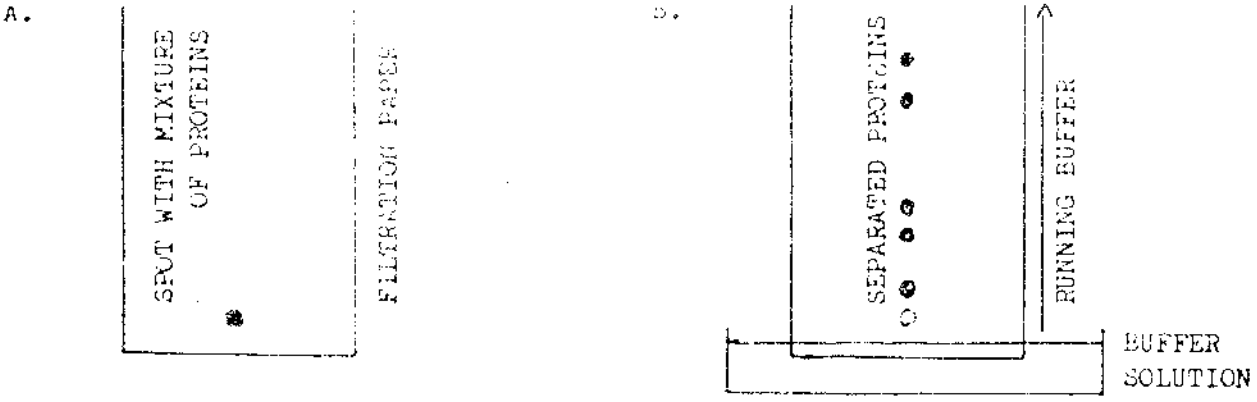

Fig 1 : chromatographical separation of a mixture of proteins.

Theoretically, complete separation of the complex protein mixture can be achieved. However, in practice, the method has not enough resolution to be able to separate all the different bird species.

2.3.2. Gas chromatography

The molecules are separated on the same principle as for paperchromatography but the mobile phase is provided by a gas (N_2). The sensitivity is determined by the lenght of the column through which the gas is chased. A longer column takes more time but the resolution becomes higher.

This technique is being used to determine the sort of flesh within the food industry with the aid of the fatty acid content of the samples. In this way, it's very easy to discriminate between cow and pig meat, whatever the presentation of it.

However, it takes a lot of skill to perform such an experiment, the apparatus is complicated and expensive and it would require

an awful lot of time to set up the protocols for all the different bird species. So, although the technique is very sensitive, it is only in some very specialised domains that its application becomes paying. More important still is the question if this method is sensitive enough to identify the birds up to the species level.

2.3.3. Electrophoresis

The resolution can be enhanced by applying the electrophoresis technique. The principle remains the same as for chromatography but the separation is mediated by the application of an electric field along the lenghth of the stationary phase.
Indeed, proteins are electrically charged in a typical way. These charges will enable the proteins to migrate under influence of an electric field. The velocity of migration is dependent on the molecular weight and on the netto electric charge of the protein. Proteins with the same charge, but a smaller molecular weight will move faster (sieving effect). On the other hand, proteins with the same molecular weight but a different netto charge will be separated because of their electrical behaviour.
Fig 2 illustrates some typical band patterns obtained with this technique on flesh samples of a few mammals. Even the untrained eye can discriminate between the different patterns.

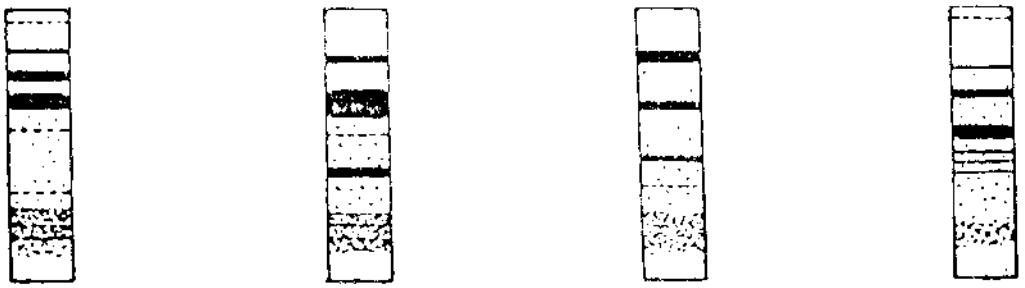

Fig 2 : electrophoretic pattern of a few mammals.

2.3.4. SDS electrophoresis

The electrophoresis technique can be further refined by a pretreatment of the proteinsolution with sodiumdodecylsulfate (SDS), an anionic detergent. SDS denaturates proteins by destroying their molecular structure into smaller pieces : the polypeptides. In this way, it is also possible to solubilise most of the heat denaturated proteins.

By coocking the sample on the beforehand at 100°C, the obtained polypeptide pattern is nearly independent of the pretreatment of the flesh remains.

Among other things, the technique is currently being used to demonstrate foreign proteins in heated meat (fig 3). It is possible to detect 0,1 % casein and 0,25 % soy.

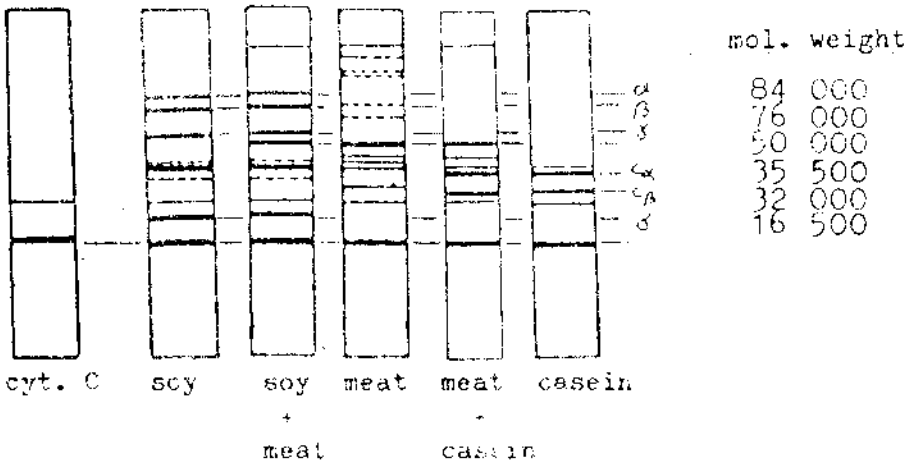

Fig 3 : SDS electrophoresis

The technique has the main advantage that it is possible to work with denaturated proteins and that the obtained band pattern has a high resolution and specificity.

2.3.5. Isoelectric focusing (IF)

By this technique the separation of the proteins is mediated through an electrically induced linear pH gradient.
At the R.U.G. Abrams et al (1983) tested AGIF (agarose gel isoelectric focusing) as a method to identify fish species.
A total of 53 species was examined. All 53 species showed a specific pattern in a highly reproducible way. Figure 4 illustrates the obtained results.

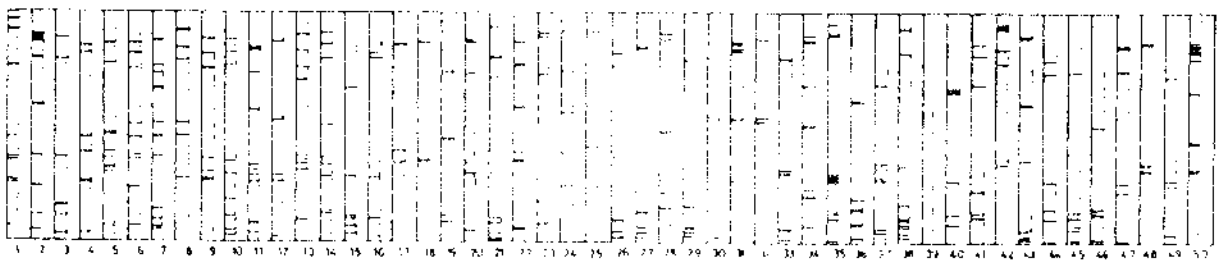

Fig 4 : Schematic patterns of fifty fish species.
(from Abrams et al (1983))

Also the effects of deepfreezing and storage at room temperature of the sample can be investigated.
IF patterns from fish extracts of the same species generally show a slight variation. Some faint bands may be slightly more pronounced or may even disappear completely.
The foregoing results illustrate that IF is a possible approach to the identification of bird species.
Still, there remain some problems : the proteins may not be denaturated, i.e. they are not to be exposed to temperatures higher than 50°C. Also, at this moment, the technique is slightly more complicated and more expensive than SDS electrophoresis.

2.4. Immunological investigation methods

The techniques currently developed are very sensitive, specific and can be applied with heat denaturated proteins. Usually, they are based on an antigen - antibody reaction.

These methods have the drawback that an antiserum against each species is required. The development and production of these antisera is very expensive and time consuming. To identify an unknown sample, each antiserum has to be tried out untill one gets a positive reaction. This is a very cumbersome way but perhaps the one to use in the future.

3. Conclusion

As a general conclusion, we may state that the best current biochemical analysis of BS remains could be provided by some electrophoresis technique. Introductory experiments will have to be performed to determine which one of these is the most suitable. During spring 1986 testings of the IEF-technique have been done at the laboratory of neurophysiology (K.U.L.) and already some preliminary positive results have been obtained.

A second phase should consist of the set up of a library with the patterns for all the bird species of a given area. This library should also provide the reference material to compare the samples.

When the system can be applied in practice, the percentage of exact identified BS samples will probably increase considerably.

If a 100 % identification should be requested, application of immunological methods should be considered.

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