and matings between positive and negative parents gave at least 50% positive offspring. The frequency of the gene Ci^A, estimated from the frequency of the phenotypes in the 277 offspring of the Table, was 0.43.

To study the constancy of the Ci(a+) antigen, from 4–6 serum samples, collected from 10 animals (6 positive and 4 negative) over almost 1 year time, were tested all at once and found to give consistent results. An animal, tested 12 h after birth, was positive. His mother was negative. This shows that the antigen Ci(a+) expresses itself at birth or soon after.

Whether this antiserum and the one described by RAPACZ et al.[6] are related, remains to be ascertained. At present it is known that they have been obtained using different antigen preparations: whole serum for the one described here and an ammonium sulphate fraction of serum for the one described by RAPACZ et al.[8,9].

Inheritance of the antigen

Type of mating	No. of matings	Offspring positive	negative	total
+ × +	26	20	6	26
+ × −	162	93	69	162
− × −	89	0	89	89

Riassunto. È descritto un antigene identificato nel siero dei bovini mediante microimmunodiffusione. I dati familiari mostrano che esso è sotto controllo genetico. L'antisiero è stato ottenuto mediante isoimmunizzazione con pool di siero bovino diluito 1:1 con soluzione tampone ed emulsionato con un egual volume di adiuvante completo tipo Freund.

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[8] The author expresses his everlasting gratitude to Prof. W. H. STONE who guided him during graduate school. He also wishes to thank Dr. J. Hirschfeld who introduced him to the study of allotypes.
[9] This work was supported by a grant from Consiglio Nazionale delle Ricerche, Italy.

Identification of Ceruloplasmin in Human Liver Cells by Fluorescent Antibodies and Absence of this Protein in Wilson Disease

Ceruloplasmin is a copper-containing blood plasma protein regulating copper metabolism in the human organism. There are data indicating that ceruloplasmin synthesis occurs in liver[1,2]. There is some evidence that in Wilson disease the patient's ceruloplasmin does not differ in general physico-chemical characteristics from that in normal subjects[3,4]. The decrease of ceruloplasmin concentration in the patient's blood is due to genetically determined inhibition of the protein synthesis. However, there has been no direct evidence so far to support this hypothesis.

The present paper reports some direct evidence of ceruloplasmin synthesis in normal human liver cells and almost entire absence of liver ceruloplasmin in Wilson disease.

Materials and methods. Slices of liver, brain, spleen, heart and kidney from a practically normal male, aged 37, who died of a sudden chest compression, were studied. The tissues of these organs were taken 2 h after registration of clinical death. Liver tissues taken in biopsy by liver puncture according to MENGINI's technique[5] were obtained from 8 patients with liver disorders of non-congenital ethiology (concentration of ceruloplasmin 45–60 mg 100 ml) as well as 3 with Wilson disease (concentration of ceruloplasmin 3–6 mg 100 ml). To obtain anticeruloplasmin serum, rabbits were immunized with human homogenic ceruloplasmin by the method described earlier[6]. For identification of ceruloplasmin in slices of various human organs COONS's indirect method was used[7]. Anticeruloplasmin serum adsorbed to remove antibodies to other plasma proteins by diazotized protein complex from a patient with Wilson disease was used as an intermediant. The adsorbtion of plasma protein antibodies of intermediant serum was complete enough since precipitation on agar gel by OUCHTERLONY's technique[8] with human ceruloplasmin, blood serum from normal subjects and those with Wilson disease showed only one arc (Figure 1). Preparations from organs obtained in autopsy and biopsy were immediately frozen and treated as described earlier[2]. The fluorescence observed was considered specific only if no fluorescence was seen in control preparations[2]. The blood serum ceruloplasmin concentration was determined by RAVIN's method[9].

Results. When studying slices of human kidney, spleen, brain and heart obtained in autopsy, no localized specific fluorescence was observed in the cells of these organs. Fluorescence was of diffuse character and slight intensity. In liver preparations, where anoxia is extremely high, specific fluorescence was seen extracellularly, i.e. in the intercellular space (Figure 2). However, certain parenchimatous cells showed sites of specific fluorescence

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(Figure 3). Blood vessels, where specific fluorescence was observed by staining with fluorescent serum containing plasma protein antibodies, showed no fluorescence when treated with anticeruloplasmin serum. This indicates that extracellular fluorescence observed in these studies is not related to the presence of blood or plasma in the preparations, but is rather due to ceruloplasmin released from the cells and accumulated in the intercellular space as a result of postmortum changes in the organ. Figure 3 shows that specific fluorescence in parenchimatous cells of human liver is accounted for by the organelles surrounding the cellular nucleus.

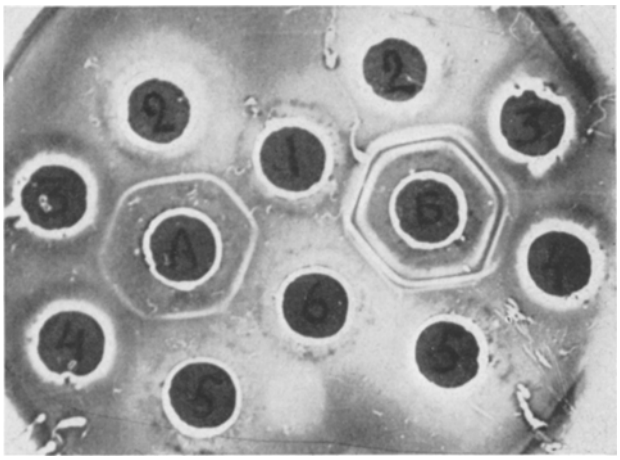

Fig. 1. Agar immunodiffusion of human ceruloplasmin, blood serum in normal subjects and in Wilson disease. (1, 6) human serum; (2, 3) normal human blood serum; (4, 5) blood serum from patients with Wilson disease; (A) dipleted antiserum; (B) native rabbit antiserum.

It is clear from Figure 4 that specific fluorescence in patients with non-congenital disease is observed in liver parenchimatous cells, the structural elements of cytoplasm with specific fluorescence surrounding the nucleus. Extracellular fluorescence was not detected. This indicates that in liver preparations obtained by biopsy, ceruloplasmin is localized in parenchimatous cells.

Figure 5 represents a picture of the liver slice from patient with Wilson disease. It is seen that there is almost no specific fluorescence, suggesting the absence of ceruloplasmin in liver cells in Wilson disease.

Conclusion. These studies demonstrate that liver is the only organ in man which is responsible for ceruloplasmin biosynthesis. This view is supported by the data obtained in the study of section material: there is no specific fluorescence in all organs except liver. In liver preparations obtained by autopsy, the capacity of cell membranes to retain ceruloplasmin seems to have been affected by changes in energy metabolism caused by

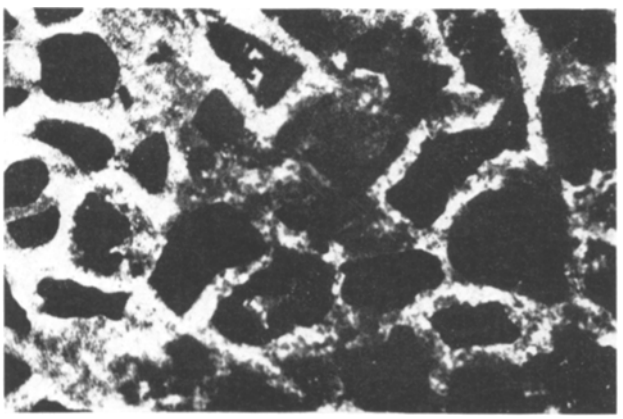

Fig. 2. Extracellular specific fluorescence in human liver slices taken in autopsy (lens × 40; ocular × 1.7; aqueous immersion).

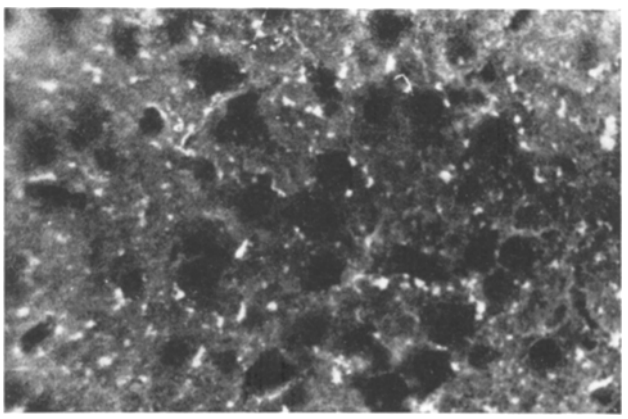

Fig. 4. Specific fluorescence in human liver slices obtained by biopsy (lens 4 × 0.75; ocular × 1.7; aqueous immersion).

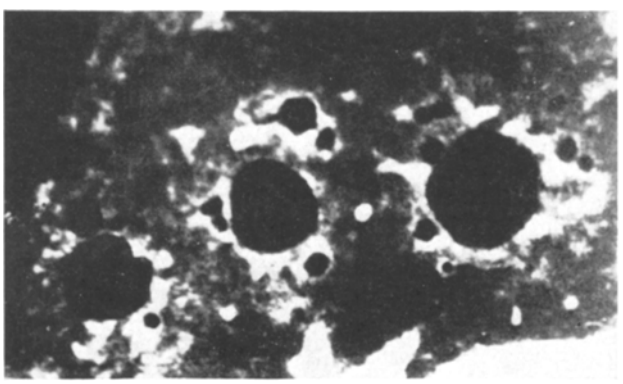

Fig. 3. Specific fluorescence in parenchimatous cells of human liver taken in autopsy (lens × 100; ocular × 3).

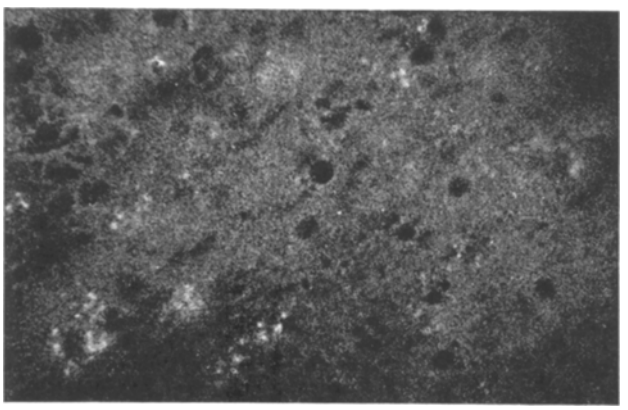

Fig. 5. Almost entire absence of specific fluorescence in the liver (biopsy) in Wilson disease (lens 40 × 0.75; ocular × 1.7; aqueous *immersion*).

anoxia. This fact appears to account for the extracellular specific fluorescence in liver, since in the study of liver preparations made in biopsy the principal specific fluorescence is detected intracellularly. However, even in the study of the liver of the man killed in accident, 2 h after death we were able to detect intracellular specific fluorescence.

In liver preparations (biopsy) from patients with Wilson disease, specific fluorescence was detected in less than 5% of all cells, whereas in liver preparations from patients with non-congenital liver disorders it was observed in more than 70% of cells. Slight fluorescence in liver preparations in Wilson disease is a direct evidence of almost entire absence of ceruloplasmin synthesis in these patients.

Almost entire absence of specific fluorescence in liver cells in Wilson disease confirms the idea that in Wilson disease there is genetically determined inhibition of ceruloplasmin synthesis.

Выводы. Установлено, что специфическая люминесценция характерная для церулоплазмина в клетках печени здоровых людей отмечается в 70% гепатоцитов, а при болезни Вильсона менее чем в 5% клеток. Это подтверждает предположение о блоке синтеза церулоплазмина при болезни Вильсона.

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The Caryotype of Some Teleostea Fish Obtained by Tissue Culture in vitro

Not a great deal is known about the fish caryology; only recently some qualitatively significant results have been obtained by using the squash technique[1,2].

Recently, through tissue culture in vitro, a tremendous increase of knowledge has become available as regards the caryotype of mammals and other higher vertebrates.

It is likely that these methods, if extensively applied to the super order of Teleostea, as we foresee, will contribute towards a solution of the numerous and complex problems of the systematics and phylogenesis of this group of vertebrates.

After modifying the in vitro culture technique used for mammals, we were able to apply it to the fish tissues[3].

This note refers to the caryotype of some of the commonest Teleostea which were used in our preliminary experiments. In the majority of plates prepared by us from fish tissues, chromosomes appeared shorter and thinner than those of mammals (Figure 3).

The sizes of chromosomes of *Carassius auratus*, for example, vary between 2.0–0.8 μ, while those of the human caryotype range from 1.5–8 μ.

These data are consistent with the histophotometric measurements of the nuclear DNA[4–6] according to which the interphasic nucleus of fish has a DNA content lower than that of mammals.

From the morphological standpoint[7] the diploid chromosome complement of *Tinca tinca* (Figure 3a) is made up of 23 (or 24) pairs with a pair marked by a peculiar hypopycnotic area on one of its arms.

The caryotype of *C. auratus* (Figure 1) shows 10 pairs of metacentric chromosomes and 42 pairs of chromosomes with a more or less terminal centromere, 6 of which seem to be acrocentric.

The same chromosome number and a similar set of chromosomes has been found for *C. carassius*.

The caryotype of *Anguilla anguilla* (Figure 4) shows 18 pairs of perfectly paired chromosomes and 1 pair (19) of dissimilar chromosomes.

The 18 pairs of matched chromosomes can be grouped into 6 pairs of roughly mediocentric chromosomes, 5 pairs of submetacentric chromosomes and into 7 pairs of roughly terminal centromere chromosomes.

The caryotype of *Scardinius erythrophtalmus* (Figure 2) is made up of 23 pairs of homologous chromosomes and 1 pair (24) of unpairable chromosomes. Though the possible presence of heterochromosomes in this species might be assumed, the fact that chromosome 24 (second) is

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7 The authors are indebted to Maria Monchietto for the diligent cooperation in organizing the caryotypes.

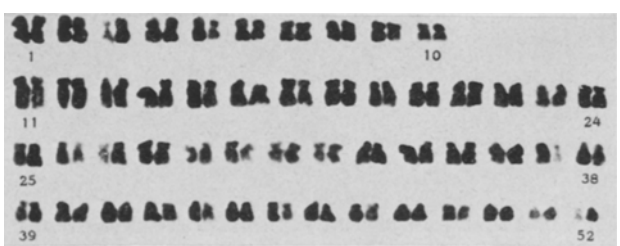

Fig. 1. Tentative pairing of the chromosomes of *Carassius auratus*.

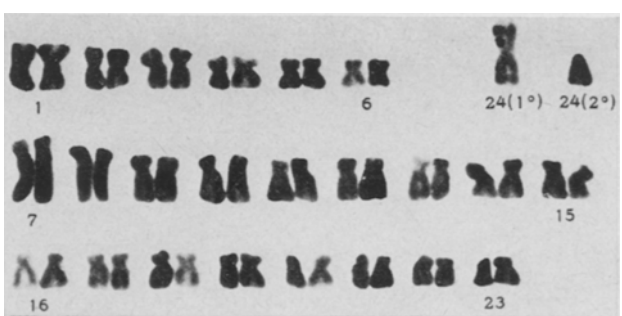

Fig. 2. Tentative pairing of the chromosomes of *Scardinius erythrophtalmus*.