medium/5% defibrinogenated plasma); the adipose cells, however, remained viable during a number of days of observation in the latter medium.

A gradient of decreasing adipose conversion and increasing cell proliferation was observed in cultures containing, respectively, 50%, 30% and 5% commercial serum (figure, b–d). In this last test medium, little adipose conversion and maximal cell proliferation were observed. Identical adipose changes were observed when commercial chicken plasma (heparinized) was used, over the same range of concentrations (undiluted, 50%, 30% and 5%), in place of commercial chicken serum. Rapid, quantitative adipose conversion was also observed when Rous sarcoma virus-infected chicken fibroblasts were cultured in undiluted commercial serum or plasma.

It was puzzling to observe that adipose conversion of fibroblasts occurred in the presence of high concentrations of commercial chicken serum or commercial plasma but occurred little, if at all, in fresh serum or heparinized or heat-defibrinogenated plasma prepared in our own laboratory. (The commercial and fresh sera did not differ, significantly, in their content of triglycerides or cholesterol). This matter was resolved when we observed that serum and plasma prepared in our own laboratory, and then allowed to age at 41.9 °C for 2 weeks, would induce adipose conversion as does commercial serum or plasma.

Adipose conversion occurred when commercial chicken serum was combined with an equal part of fresh serum indicating that the commercial serum contained an initiator, rather than lacked an inhibitor, of the change. Adipose conversion did not occur when an ultrafiltrate of commercial serum (20,000 molecular weight cutoff) was combined with an equal part of fresh serum, indicating that the active principle in commercial serum is a macromolecule or is bound to a macromolecule.

Adipose conversion of chicken fibroblasts by serum or plasma, as described here, appears to be very similar, if not identical, to the spontaneous adipose conversion that has been described with sublines of 3T3 cells. Our results indicate that chicken fibroblasts, like some 3T3 sublines, are susceptible to this change. Unlike the 3T3 systems, however, adipose conversion of chicken fibroblasts is rapid (2 days) and quantitative and does not require cell confluency. Indeed, adipose conversion of chicken fibroblasts by undiluted chicken serum or plasma causes cell proliferation to cease at low (subconfluent) culture densities.

Adipose conversion in the 3T3 systems, as noted earlier, is inhibited by bromodeoxyuridine at a concentration of 5×10^{-6} M. Adipose conversion of chicken fibroblasts was not, on the other hand, affected when these cells were allowed to divide 5 times in growth medium containing 10^{-5} M bromodeoxyuridine, before being passaged in the same and then exposed to undiluted serum containing the above concentration of the drug. It is possible that the rapidity of the adipose change in the chicken system precludes an inhibitory effect of bromodeoxyuridine. On the other hand, it is possible that the adipose change that we describe here represents a form of lipid degeneration, rather than representing true adipocyte differentiation. In either case, we have demonstrated that the ageing of serum or plasma generates a principle that is capable of causing adipose change in cultured fibroblasts. Further study of this principle and of the change that it causes may provide worthwhile information regarding control of cellular differentiation or the initiation of a specific form of cellular damage.

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An actin-destabilizing factor is present in human plasma[1]

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Summary. Plasma and serum of humans or experimental animals contain a factor which destabilizes F-actin. The factor has no DNAse or thrombin activity and after incubation with F-actin does not modify the position of the actin band on a SDS polyacrylamide gel. Hence it probably depolymerizes F-actin.

Human anti-actin autoantibodies (AAA) have been used in several laboratories as a tool for the study of actin distribution in cells under different conditions[2–5]. Preliminary work from this laboratory had indicated that there is a difference in the pattern and intensity of AAA stainings after using whole sera or affinity column purified antibodies. Here, we report that the plasma and serum of humans or experimental animals (e.g. rabbit, rat, guinea-pig) contain an actin-destabilizing factor responsible for this staining difference.

We used the sera of 2 patients with chronic aggressive hepatitis having a titer of 1/1280 and 1/640 respectively when tested on rat intestinal smooth muscle. The sera were passed on column of CNBr activated sepharose[6] or on a glutaraldehyde immunoabsorbent[7] covalently linked with rabbit skeletal muscle actin[8], followed by elution of the antibody at pH 2.7. The specificity of these antibodies was tested by immunodiffusion, immunoelectrophoresis and immunofluorescence as described previously[9,10]. Mouse fibroblast cultures were prepared from 12–14-day-old embryos of Swiss albino CR-1 mice[11]. Secondary cultures were always used. The cultures were fixed and stained when non-confluent and at confluence. We examined also frozen sections (4 μm thick) of normal rat liver. For immunofluorescent staining, cells on glass coverslips were fixed 30 sec

in absolute ethanol and frozen sections of the tissues were either left unfixed or fixed for 5 min with acetone at −20 °C. Cells and tissues were then incubated first with plasma, serum or a purified serum fraction for 15 min, then washed with phosphate-buffered saline (PBS) and incubated with purified AAA for 15 min, washed 3 times in PBS then incubated with fluorescein conjugated IgG fraction of goat anti-human IgG antiserum (Miles Seravac, Lausanne, Switzerland). After rewashing in PBS and mounting in 90% glycerol in PBS, the level of fluorescence was compared with that found in control preparations treated with PBS instead of the factor containing fractions. In some coverslips, AAA staining was followed by incubation with a rabbit serum containing antibodies against smooth muscle myosin from human uterus[12], followed by rhodamine conjugated IgG fraction of goat antirabbit IgG antiserum (Behring Werke AG, Marburg/Lahn, West Germany).

Figure 1 shows that incubation of the cells with human serum abolishes AAA staining, but, in the same cell, leaves unaffected the staining with antimyosin antibodies. This destabilizing activity of plasma or serum (as well as that of the partially purified fraction) was always higher in non-confluent than in confluent cells. Similarly, in frozen sections of rat liver, the activity of plasma or serum was stronger in hepatocytes (pericanalicular web) than in smooth muscle of bile ducts or vessels.

In order to study directly the action of serum on actin filaments, we incubated for periods from 1 to 5 min a drop of rabbit striated muscle F-actin with 1 drop of serum (or 1 drop of PBS as control) and then negatively stained with 1% uranyl acetate these drops on a formwar-filmed grid. As figure 2 shows, the incubation with PBS did not modify the shape of actin filaments, while even 1 min incubation with serum resulted in a practically complete disappearance of actin filaments.

In order better to characterize the actin-destabilizing factor, we used 3 purification steps starting from 20 ml serum: 1. 50% saturation of ammonium sulfate was necessary to recover the factor; 2. the 50% precipitated fraction was then dissolved in 5 ml of 0.0175 M potassium phosphate buffer, pH 8.0, dialyzed against this buffer, clarified by centrifugation and charged (5 ml, 35 mg proteins/ml)[13] on a 1.7×25 cm DEAE cellulose column (DE-52, Whatman Biochemicals Ltd, Maidstone, England) equilibrated with the same buffer[14]. A linear ionic strength gradient in potassium phosphate from 0.0175 M up to 0.3 M was used. 2 major peaks were recorded (at 280 nm) and the second one contained the factor; 3. the DE-52 factor containing fraction (2 ml, 25 mg/ml) was dialyzed against PBS and applied to a G200 Sephadex column (2.4×100 cm, Pharmacie Fine Chemical AG, Zürich, Switzerland). The factor was recovered at the end of the last peak elution: this suggests a molecular weight between 30,000 and 100,000. The fraction recovered (figure 3,a) had the same action as the total serum or plasma on AAA staining of fibroblasts or liver and on F-actin negatively staining.

4 additional experiments were performed with the factor

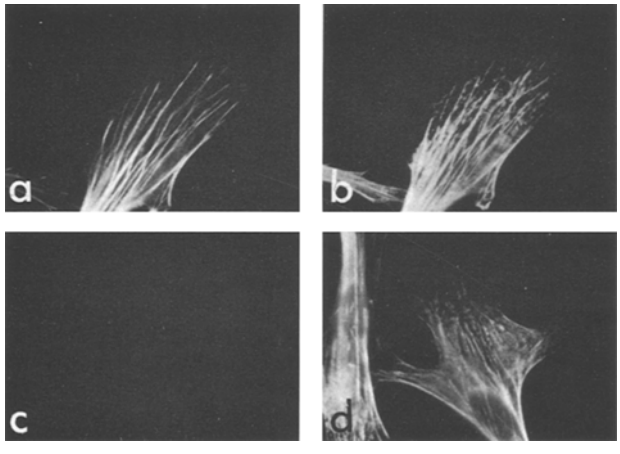

Fig. 1. Inhibition of AAA staining by normal human serum. Cultivated fibroblasts were stained first with purified AAA followed by fluorescein conjugated anti-human IgG, then with antimyosin antibodies containing serum (AMA) followed by rhodamine conjugated antirabbit IgG. *a* AAA staining; *b* AMA staining of the same cell; *c* incubation with human serum followed by AAA staining; *d* AMA staining of the same cell. × 700.

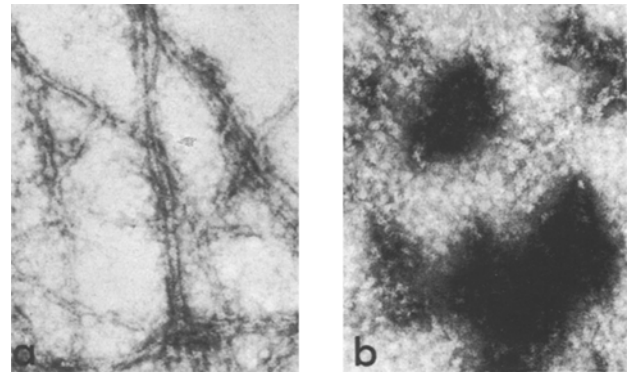

Fig. 2. Disappearance of actin filaments (negative staining) 1 min after incubation in normal human serum. *a* F-actin incubated in PBS; *b* F-actin incubated in normal human serum. × 107,000.

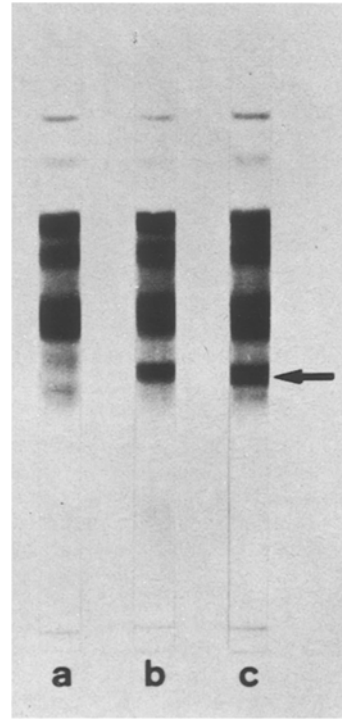

Fig. 3. SDS polyacrylamide gels of: *a* actin-destabilizing factor-rich fraction after purification on G200 Sephadex column; *b* the same fraction incubated for 15 min over coverslips with cultivated fibroblasts; *c* as *a*+3 µg of rabbit skeletal muscle actin. The arrow points the band of actin which clearly comigrates with the new band in *b*.

containing fraction: 1. confluent cultivated fibroblasts on glass coverslips were fixed for 30 sec with ethanol, rinsed 3 times in PBS and then incubated with the factor containing fraction (50 µl, 500 µg/ml per coverslip). The incubation drop was recovered 15 min later, boiled for 3 min in sample buffer and applied on a 10% SDS polyacrylamide gel[15]. As a control, the same incubation was made with PBS. A new band comigrating with actin was clearly visible only on the gel of the sample incubated with the factor (figure 3,b). 2. The factor containing fraction (200 µl) was incubated first with trypsin (2 µl, 5 mg/ml in PBS, Worthington Biochemical Corp., Freehold, N.J., USA) for 1 h at room temperature, then trypsin inhibitor was added (Soyabean trypsin inhibitor, 2 µl, 10 mg/ml distilled water, Worthington Biochemical Corp.; coverslips of cultivated fibroblasts were treated with the trypsinized fraction followed by staining with AAA. The trypsinized fraction did not abolish AAA staining. 3. The factor-containing fraction was incubated overnight with F-actin from rabbit skeletal muscle, then boiled for 3 min in sample buffer and applied on a 10% SDS polyacrylamide gel. No changes in the position of the actin band were seen on the gel when compared to F-actin non-incubated with the factor. 4. We tested DNAse activity (by checking on agarose gel electrophoresis the nicking of supercoiled r-DNA plasmid[16]) and thrombin activity[17,18] of the fraction containing the factor; in both cases the tests were negative.

These results show that human plasma and serum (as well as the other plasma or sera tested) contain a factor which destabilizes F-actin. Since it does not alter the migration of actin on a SDS polyacrylamide gel, it is probable that the factor depolymerizes actin. The possibility of an actin-depolymerizing activity of plasma was briefly raised recently[19]. Further studies are on the way in order better to characterize the nature of this actin-destabilizing factor and to study its possible function.

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Influence of the earth's magnetic field on the comb building orientation of hornets

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Summary. Introduction of a magnetic field that counteracts the vertical component of the earth's field, thereby creating a total 'zero field' is lethal to juvenile hornets and completely disrupts the comb-building orientation of adult hornets. This suggests that the terrestrial magnetic field is the main guideline for vespan building orientation.

It has been shown by Martin and Lindauer[1] that the earth's magnetic field affects the orientation of insects. The influence of additional magnetic fields on the building orientation and behavoir of hornets *(Vespa orientalis)* has been reported by the present authors[2,3]. Ishay and Sadeh[4] noted that adult hornets placed in a centrifuge built their combs in the normal vertical direction, although the resultant of the gravitational and centrifugal forces was directed at 45° from the vertical; juvenile hornets placed in the same centrifuge built their combs in the direction of the resultant force. These findings suggested that adult hornets develop an orientation mechanism that helps them build in the right way even under changed gravitational fields. The influence of the horizontal component of the terrestrial magnetic field on randomly moving hornets is averaged to zero, which indicates that vespan building orientation is influenced only by the vertical component of the terrestrial magnetic field.

The aim of the present study was to investigate the influence of the earth's magnetic field on the comb-building orientation of hornets, which in nature as well as in artificial breeding boxes (ABBs) is almost invariably in the vertical direction[2].

Materials and methods. Hornet workers *(V. orientalis)* maintained in ABBs in groups of 10–20 individuals were placed both inside a previously described solenoid[2,3] as well as at some distance from it in the same room (control). Experiments were conducted with adult worker hornets more than 1-day-old and juvenile hornets of lesser age. The hornets were provided with the usual food and building material[2]. The solenoid was placed vertically on 2 timber slabs to allow air circulation through its interior which housed four ABBs during each experiment. The maximum current in the solenoid's coil was 37.2 mA (inverted natural field). The heat power dissipated did not exceed 0.04 W and no