quently no volumetric changes could be detected. From the first to the second month, the liver cells progressively resumed their DNA synthesis activity while the mitotic recovery was still lacking. The outcome of this is the appearance of giant cells as the regenerative process tries to compensate for the cell necrosis induced by the irradiation.

The high frequency of aneuploids deserves special comment (Table II). The lateral spread of the histogram accompanied by a disappearance of $2n$ nuclei and a progressive decrease in the frequency of $4n$ nuclei suggest that the aneuploidy is not due to abnormal mitosis with unequal distribution of chromosomes between the daughter cells, but more likely results from asynchronous endoreduplication of chromosome sets. The constancy in frequency of the binucleates shows that the giant cells are not produced as a consequence of mitosis with a common spindle in these elements. Micronuclei and bizarre nuclei were observed 2 months after injection, when the liver cell population was almost entirely composed of polyploids and aneuploids. The presence of these aberrant elements indicates that few cells resume their mitotic activity although faulty mitosis is the result. Again, the absence of mitotic figures together with the observation that the % of micronuclei is kept constant from the moment of their appearance throughout the whole period of observation, suggests that those cells that regain their divisional capacity died after a few divisions, sometime between 30 and 64 days after the gold administration (Table I).

The occurrence of polyploidy and aneuploidy and the progressive increase in the frequency of chromosome aberrations in aging mammal liver is a fact already established and well documented[14–17]. Irradiated livers look 'older' when compared to the same organ of the intact animals of the same age. Polyploidization and aneuploidization in normal livers can be understood as a phenomenon peculiar to reverting post-mitotic cells in which the usual process of cell turnover by means of mitosis does not take place at a proper rate and where the 'physiological' cell loss is deficiently compensated by increasing the size of the remaining elements. By inducing necrosis and concomitantly blocking mitosis almost indefinitely, what radiation did in our material was to accelerate a physiological process or, to say it in a rhetorical way, to condense the biological time of the system under consideration. It is highly probable that polyploids and aneuploids, due to their unbalanced chromosome sets, are defective elements within the whole system and are unable to cope properly with the metabolic tasks that are the burden of the liver cells. As a matter of fact, KOLETSKI[2] has already demonstrated by means of the bromosulphalein test that these giant cells are functionally incompetent.

If we define aging as a progressive and irreversible disruption of form and impairment of function that leads to gradual decrease in the resistance to environmental stress, we might perhaps say that as the number of these giant elements increases the liver 'ages'. The experimental results discussed above and expressed in the histogram and Tables I and II, confirmed previous observations by others, revealed some new aspects of the irradiation effects, and suggest the following conclusions: (a) At high doses of radiation both the DNA synthesis and mitosis are inhibited. (b) The DNA synthesis resumes much sooner than the mitosis activity after irradiation. A number of cells never recover their divisional capacity, indicating that the DNA synthesis and mitosis although related to each other are controlled by different and independent mechanisms. (c) Regenerative effort in a cellular system where mitosis is inhibited leads to progressive polyploidization and aneuploidization. (d) Polyploidy and aneuploidy in our material seem to be due to synchronous and asynchronous endoreduplication of chromosome sets and not to aberrant mitosis. (e) Cells that recover their divisional capacity following high doses of radiation seem to die after a few divisions. (f) The morphological and quantitative similarities between radiation induced changes and aging changes in rat liver, indicate a common biological mechanism underlying both processes. Polyploids and aneuploids being physiologically incompetent cells, we suggest that their increased frequency with time is the 'cause' of aging in livers of rats.

Résumé. On a etudié l'induction de polyploidie et aneuploidie dans les foies des rats à la suite d'injection intraveineuse d'or colloidal radioactif. On souligne l'analogie entre ce phénomène et l'observation de phénomènes semblables faites sur les foies normaux d'animaux âgés. On suggère une relation 'causale' entre l'augmentation de la fréquence de ces éléments polyploidiques et aneuploidiques et le vieillissement hépatique.

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Fluorescein Staining of Guinea-Pig Lymphocytes Induced by *Echis colorata* Venom

In the course of a study[1] on the action of *Echis colorata* venom on the blood-brain barrier, it was found to affect the permeability of guinea-pig lymphocytes. Guinea-pigs, weighing 250–300 g, were injected intracardially with a mixture of 100 μg (protein content[2]) *Echis colorata* (EC) venom and 25 mg fluorescein (Fluorescite, Moore Kirk Laboratories Inc., Worcester, Mass.) in 1 ml of saline solution. Such animals died within 15–30 min. Blood samples were obtained by cardiac puncture 5, 10 and 15 min after injection of the mixture. Figure 1 shows fluorescein-stained lymphocytes from an animal injected with venom and fluorescein. The fluorescence-inducing action of the venom was manifest at all 3 time intervals. Fluorescein-staining of lymphocytes was observed in all animals in which lethal venom doses, i.e. a minimum of 20 μg of venom, were administered. It should be noted that in the control non-venom-treated animals the platelets became strongly fluorescent (Figure 2). The absence

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of platelets in the venom-treated animals is consistent with the previously reported severe thrombocytopenia occurring in EC venom-inoculated animals[3].

The intravenous injection of boiled venom[3], which possessed phospholipase A activity but was devoid of protease as tested on gelatin, did not induce fluorescein staining of the lymphocytes. Each of the 2 protease (gelatinase) fractions obtained by elution from a starch gel electrophoregram (Figure 3) was able to induce strong lymphocyte fluorescence, whereas the procoagulant fraction which was devoid of gelatinase had no such effect.

The action of the venom and its fractions was studied in vitro using non-washed guinea-pig leucocytes, separated by the use of polyvinylpyrrolidone and ammonium chloride[4]. $3 \cdot 10^7$ leucocytes were incubated at 37 °C for 15 min with 100 μg of EC venom and 1 mg of fluorescein in a final volume of 1 ml of phosphate-buffered saline ($1.33 \cdot 10^{-3} M$ phosphate 0.88% NaCl pH 7.4). Smears were prepared from the sediment obtained by centrifugation for 3 min at 135 *g*. The venom induced strong fluorescence of the lymphocytes, whereas control fluorescein-treated leucocytes were negative. Saline washing of the separated leucocytes, prior to their contact with fluorescein, made them susceptible to fluorescein staining also in the absence of venom. Circumstantial evidence for penetration of the fluorescein in the venom-treated leucocytes was furnished by the negative result obtained when albumin-bound fluorescein prepared according to Riggs et al.[5] was used, presumably the large size of the molecule preventing its entry into the cell.

Neither the boiled EC venom nor its procoagulant fraction were able to induce lymphocyte fluorescence. That the phospholipase A was not involved in the fluorescence-inducing action of the whole venom is also clear from the absence of phospholipid splitting, examined by silicic acid paper chromatography[7], in the leucocytes subjected to the whole venom. Each of the 2 EC protease fractions, on the other hand, produced the fluorescent effect. If the proteases themselves and not an impurity induce the fluorescein penetration into the guinea-pig lymphocyte, their action appears to be rather specific for the EC venom, since 2 other snake venoms studied (*Naja naja* and *Vipera palestinae*, both having strong proteolytic activity) were not able to induce lymphocyte fluorescence in vivo. It is noteworthy that also trypsin, 200 μg/$3 \cdot 10^7$ cells, had no fluorescence-inducing activity. The specificity of the EC venom proteases is also illustrated by the observation that their fluorescence-inducing activity is not abolished by soya-bean trypsin inhibitor or diisopropylfluorophosphate. It seems pertinent that these protease inhibitors do not affect the EC venom protease activity on gelatin either.

The significance of the permeability-enhancing effect of the EC venom for its toxic action is not clear. It is not impossible that the venom proteases may facilitate the penetration of venom toxins through cellular membranes[8].

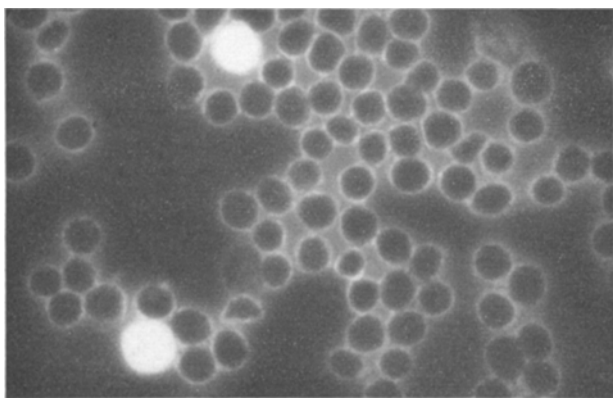

Fig. 1. Fluorescein-stained lymphocytes from a guinea-pig injected with EC venom-fluorescein mixture.

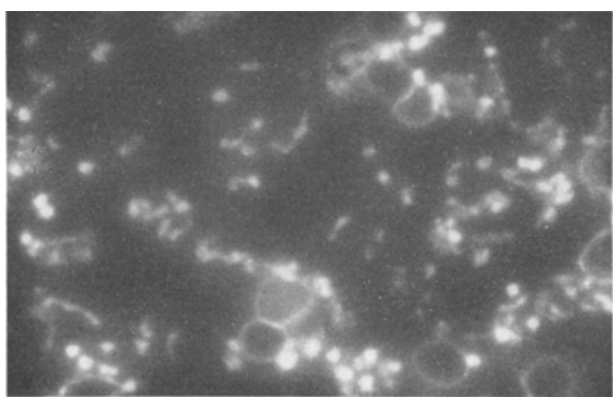

Fig. 2. Blood smears from control guinea-pig injected with fluorescein only. Note fluorescent platelets and absence of lymphocyte staining. (The lymphocytes were identified by light microscopy.)

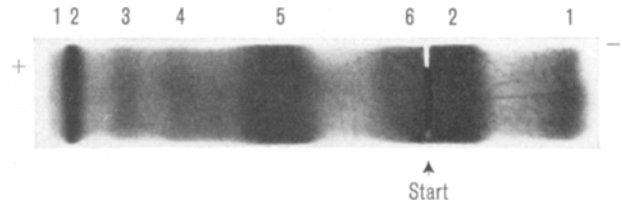

Fig. 3. Starch gel electrophoresis of *Echis colorata* venom[6]. 6 anodic and 2 cathodic fractions were obtained. Fractions + 1, + 2 (combined) and − 2 showed protease activity. The strongest coagulase activity was found in fraction + 5.

Résumé. Les lymphocytes de cobayes inoculés avec du venin de serpent *Echis colorata*, additionné de fluorescéine, acquièrent une forte fluorescence. Le même effet du venin est observé avec des lymphocytes traités in vitro. Les fractions protéolytiques du venin sont responsables de ce phénomène, alors que la phospholipase est sans effet.

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