Electrophoretic Study of the in vitro Binding of 3,4-Benzpyrene to the Plasma Proteins

This experimental work has been performed to study both the influence of a previous lipid-extraction of the plasma and the extraction by solvents after the interaction of hydrocarbonplasma on the distribution of benzpyrene between the different plasma fractions.

The lipid-extraction of the plasma was done according to WATERS[1]: 1 ml of 2-octanol were added to 10 ml of fresh human plasma in a glass-stoppered test-tube. After 1 h of active shaking, it was left overnight at 4 °C. Then the aqueous layer was separated by centrifugation, the alcohol layer was discarded and the remaining traces of 2-octanol were removed by extraction with ether, removal of the ether layer, and finally evaporation of the residual ether at low pressure at 37 °C.

For the interaction benzpyrene-plasma, 2 ml of pooled human plasma, or lipid-extracted plasma were incubated 2 h at 37 °C with the residue of a vacuum evaporation of a ^{3}H-labelled 3,4-benzpyrene benzene solution, equivalent to 4 μg of benzpyrene with a specific activity of 27 mC/mM. After the incubation the plasma was filtered through paper and a sample was used for a starch gel electrophoresis. For the electrophoresis 13% hydrolyzed potato starch in phosphate buffer pH 6.8 (27.7 ml 0.5M KH_2PO_4 + 24.1 ml of 0.5M Na_2HPO_4 brought to 1 l with distilled water) was used. Applying a voltage gradient of 7–9 V/cm, the albumin fraction migrates approximately 0.8–1 cm/h. Because of the heat released during the electrophoresis, this operation was carried out into the ice box in order to avoid any thermal denaturation of the proteins.

When the electrophoresis was ended the gel was sliced into 3, horizontally. One of the slices was stained with water soluble Nigrosine (0.1% solution in 50% acetic acid) overnight and the dye which was not bound to protein was removed by washing the gel with 60% ethanol. When it was necessary to spot the lipo-proteins, another slice was stained with a saturated sudan black solution in dioxane, diluted with a mixture of dioxane-ethyleneglycol (1:4) and the excess of dye was removed with 60% ethanol. The third slice was used for the radioactivity measurement. For this purpose it was cut vertically into 2.5 mm sections. In the case when the proteins were not treated by solvents, the sections were dried under vacuum before the counting. When they were extracted by solvents, before the sections' cutting the whole slice was processed according to HEIDELBERGER[2]: washed 3 times with methanol-water-acetic acid (5:5:1) for 1 h, twice in absolute methanol for 10 h, and 4 times in benzene for 3 h. After this treatment the transparent gel was cut and the sections added to 10 ml of scintillation solution, which contained 40 mg of 2,5-diphenyloxazole and 1 mg of 1,4-bis-2(5-phenyloxazolyl)-benzene in toluene and counted in a liquid scintillation spectrometer.

Figure 1 shows the radioactivity distribution in whole plasma, with and without solvents treatment after the electrophoresis, as well as the nigrosine stain of the proteins.

Figure 2 gives the same values for whole plasma and lipid-extracted plasma, being both treated by solvents after the electrophoresis. Also, the nigrosine and sudan black stains are included.

Finally, Figure 3 shows the radioactivity distribution in both whole plasma and lipid-extracted plasma without the solvents treatment.

In all these cases a general similar pattern of benzpyrene binding has been observed: the highest amount

[1] L. L. WATERS, Yale J. Biol. Med. *37*, 204 (1964).
[2] CH. HEIDELBERGER and C. W. ABELL, Cancer Res. *22*, 931 (1962).

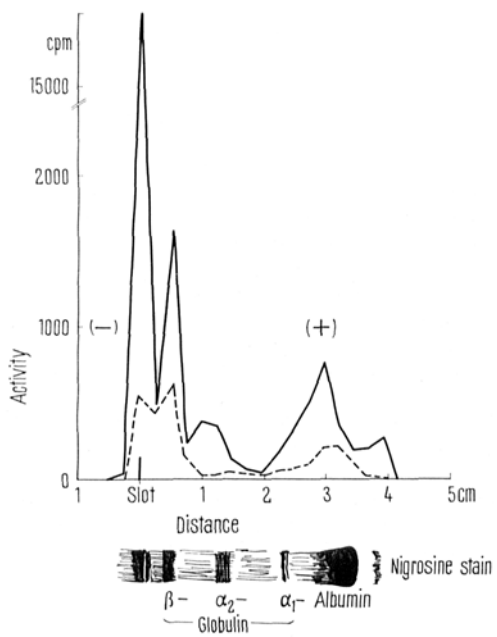

Fig. 1. Radioactivity distribution in the starch gel electrophoresis with (----) and without (——) solvents treatment.

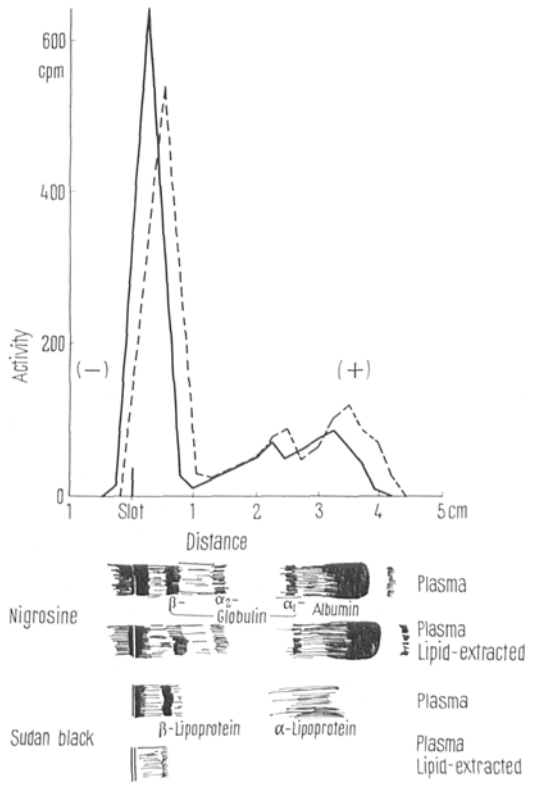

Fig. 2. Influence of the lipids extraction on the radioactivity distribution in the different plasma fractions. ---- plasma lipid-extract, —— plasma.

to the β-globulin fraction and also a significant quantity to the albumin. The solvents treatment after the electrophoresis eliminates most of the activity present in the slot (slow moving lipoproteins) and also a part of that corresponding to the different fractions of plasma proteins. The fraction of the original activity that remains after this treatment is approximately $^1/_{40}$ for the slot, $^1/_3$ for the β-globulin fraction and also $^1/_3$ for the albumin (Figure 1). As can be seen in Figure 2, the radioactivity distribution is the same in whole plasma and in lipid-extracted plasma after the treatment with solvents. If the plasma has been extracted with solvents previously to the electrophoresis (WATERS), it is a similar pattern of radioactivity distribution, in both cases, with a new post-electrophoresis treatment (HEIDELBERGER) or not.

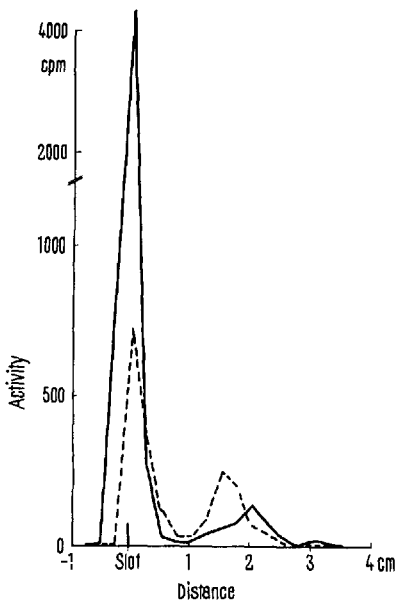

Fig. 3. Radioactivity distribution in the starch gel electrophoresis without solvents treatment. ---- plasma lipid-extracted, —— plasma.

These experimental values demonstrate that most of the benzpyrene is bound or dissolved into the β-lipoprotein fraction and the high activity observed in the proximity of the slot suggest that, because of this hydrocarbon association, the lipoprotein mobility changes are slowing down. The solvents treatment eliminates this activity, giving a residual benzpyrene assumed to be bound to the other different protein fractions[3].

Zusammenfassung. Es wird gezeigt, dass die Verteilung von Benzpyren auf die Proteine von octanolextrahiertem Plasma einen Einblick in die bei der Plasmabindung von Substanzen vorkommenden Konkurrenzreaktionen erlaubt.

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[3] This research work has been performed at the Institut du Radium (Paris) during the tenure of a Bourse Joliot-Curie.

Trasmissione del tumore di Yoshida con estratti acellulari del medesimo

Sono molte, come è noto, le difficoltà relative alla dimostrazione della etiologia virale delle neoplasie. Tali difficoltà, di ordine prevalentemente metodologico, sono soprattutto dovute alla necessità di ottenere la riproduzione della neoplasia in assenza degli elementi cellulari specifici che entrano nella sua costituzione.

Con riferimento al tumore di Yoshida, sia esso in forma ascitica, sia esso a struttura solida, le cennate difficoltà persistono a tutt'oggi, malgrado le importanti osservazioni in proposito di HAMAZAKI e coll.[1], i quali sono riusciti ad ottenere la riproduzione delle forme solide della neoplasia con la somministrazione ai ratti di pappe tumorali.

SCHMIDT[2], in esperienze di controllo eseguite con tecniche similari a quelle degli Autori giapponesi, è riuscito ad ottenere gli stessi risultati. Ciò malgrado, esaminando questi ultimi criticamente, è pervenuto alla conclusione che non si può escludere in modo certo, nella riproduzione del tumore, l'intervento di elementi cellulari sopravvissuti alla digestione gastro-intestinale.

Pertanto, ci siamo proposti di esaminare, sulla base dell'esperienza già in passato acquisita da uno di noi, la possibilità di indurre il tumore di Yoshida con estratti tumorali sicuramente acellulari, nel tentativo di ottenere valida dimostrazione della etiologia virale di tale forma neoplastica.

Abbiamo, a tal fine, utilizzato del tumore di Yoshida in forma ascitica ottenuto da ratti adulti, maschi e femmine, al 6°–7° giorno dall'inoculazione. Il liquido ascitico, in quantità di ml 180 circa, è stato sottoposto a centrifugazione a 1000 *g*/10 min, onde ottenere le cellule tumorali, che sono state sottoposte ad omogeneizzazione in omogeneizzatore meccanico MSE a 10.000 giri. L'omogenato, diluito con ml 100 circa di plasma dello stesso tumore ascitico, è stato centrifugato a $13.000 \cdot g/20$ min. Al termine di tale operazione sono stati prelevati, con molta cautela, circa i tre quarti del liquido sovranatante mediante pipettamento ed evitando ogni scuotimento delle provette. Tale sovranatante è stato ancora centrifugato a $105.000 \cdot g/60$ min, allo scopo di ottenere, in corrispondenza del sedimento, il maggiore arricchimento possibile del presunto agente etiologico. Il sedimento, equivalente a circa ml 1,5 in volume, è stato diluito con circa ml 6 di sovranatante della stessa centrifugazione, e quindi omogeneizzato come già indicato per le cellule. Gli accertamenti microscopici condotti su strisci del sedimento, hanno consentito di accertare l'assenza di elementi cellulari.

[1] Y. HAMAZAKI, K. OGAWA, I. ARIKI and M. KONDO, Proc. Japan Acad. *31*, 480 (1955).

[2] F. SCHMIDT, *Krebs, Virus und Induktor* (Akad. Verlag, Berlin 1960).