Study of the fate of DNA immediately after its administration in mammals is important since specific biologic effects may be induced very early, namely, transformation, infectivity, and neoplasia.

Generally, papers published in this field (8, 24, 27) are based upon methods which do not always allow distinction between the initial macromolecules injected and their degradation products. Since it is known that biologic activity of DNA is related to its degree of polymerization (6, 13, 16, 21, 29), this parameter has been measured directly by viscosimetry after i.v. or i.p. injection of DNA. Parallel chemical determinations were also made.

In an attempt to correlate the DNA catabolism and the DNase plasma activity, these experiments were done on individual rabbits, in which the blood enzymatic activity varied greatly from one animal to another, and on different inbred strains of mice, each strain having a constant and genetically conditioned DNase activity different from the other and little variation among animals.

Moreover, the DNA catabolism has been followed in the presence of substances (Na-citrate, MG) which are known to inhibit DNase activity (12). The results indicate that the period throughout which an exogenous DNA would maintain in the blood a physical state compatible with any specific biologic potency is very short—not more than a few min; they also show that the plasma DNases play a nearly exclusive part in the initial fate of DNA, only a very small fraction of native macromolecules diffusing out of the blood stream. Some of these experiments have been previously reported briefly (19).

MATERIAL

DNA.—DNA extracted from calf thymus according to the method of Kay et al. (9) (Sample a) had M.W., $5.2 \times 10^6$; $[\eta]$, 3200; $\epsilon_P$, 6800; according to the method of Signer and Schwander (23) (Sample b), M.W., $6.5 \times 10^6$; $[\eta]$, 6200; $\epsilon_P$, 6900; and according to the method of Aubin et al. (1) (Sample c), M.W., $6 \times 10^6$; $[\eta]$, 5200; $\epsilon_P$, 6600.

M.W. was measured by light scattering with an apparatus of the C.R.M. Strasbourg type according to Wippler and Scheibling (28). DNA solutions were purified by centrifugation for 5 hr at 30,000 × g (Servall, refrigerated). Phosphorus and protein contents were determined according to Dryer et al. (4) and Lowry et al. (18), respectively; all the samples contained <0.5 % protein. Viscosimetric determinations were done as described below.

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Methyl Green (Merck).—Solutions in 0.14 m NaCl were purified by several chloroform washings.

DNA-Methyl Green Complex (DNA-MG) Solution.—DNA (2 mg/ml), 45 ml; 0.2% MG, 45 ml; Tris-HCl buffer, 0.4 m, pH 7.4, 10 ml, were mixed.

METHODS

1. Chemical Determinations of Insol-DNA and Sol-DNA in Blood Plasma.—These were done according to a slightly modified Schmidt-Thanhhauser method (22). Plasma was treated with twice its volume of PCA (9%) at 4°C; after centrifugation, sol-DNA was found in the supernatant. Precipitates which contained insol-DNA were washed with PCA (9%), hydrolyzed twice for 30 min in 1 volume of PCA (0.5 m) at 70°C, and then washed with PCA (0.5 m). Analysis of deoxyribose was made in each fraction by the Dische-Burton method (2), pure deoxyribose (National Biochemical Corporation) being used as a standard.

2. Treatment of Animals.—We used 3-kg rabbits of the strain “Fauve de Bourgogne,” 6–8 months old, whose plasma DNase activity varied from undetectable to about 0.20 KU, and 3 strains of mice with a wide range of DNase activity: strain C3H (0.12 ± 0.01 KU); strain Swiss (0.21 ± 0.02 KU), whose characteristics are described elsewhere (3); and strain XLII (0.29 ± 0.02 KU), raised at the animal department for 22 generations.

a) DNA Administration i.v.—In 1 experiment, 4 rabbits having comparable plasma DNase activity (0.11 KU) were chosen; they received 3, 5, 10, and 20 mg of DNA. In another experiment, 21 rabbits whose plasma DNase activity varied from 0.03 to 0.18 KU received 10 mg each of DNA. Individual blood sampling was done at regular intervals by heart puncture. DNase activity did not vary from one sample to another.

Twenty-five to 30 mice were selected in each strain; each mouse received 200 µg of DNA; groups of 5 animals were sacrificed at chosen intervals; pooled blood samples were gathered to determine DNase activity either in heparin or trisodium citrate (0.2 ml of a 0.33 M solution) or for viscosimetric or chemical determination.

To test the role of DNase inhibitors, 2 series of experiments were designed: in the 1st one, 3 rabbits received 10 i.v. administrations of 30 µmoles of trisodium citrate over 60 min, 10 mg of DNA being injected immediately after the 1st citrate injection; in the 2nd one, 5 to 7 ml of a solution of 0.2% MG were injected i.v.; immediately afterwards, 11–13 ml of DNA-MG complex were given, and finally a slow perfusion of MG (20 mg in 15 min) was made to decrease the rate of complex dissociation by keeping the free dye concentration as high as possible.

DNA solutions were injected in the marginal vein of the rabbit ear and in the tail veins of mice. DNA did not elicit any symptom of toxicity. MG is markedly toxic.

b) DNA Administration i.p.—Swiss mice were used, each animal receiving 0.5 ml of a solution of DNA, 2 mg/ml, or 1 ml of a solution of DNA-MG previously described. Immediately after injection and at regular intervals blood samples were removed from the retro-orbital sinus.

3) Determination of Plasma Neutral DNase Activity.—This was done according to the method of Kurnick (10); a plasma containing 1 Kurnick unit/ml has the same enzymatic activity as a crystalline pancreatic DNase Worthington solution of 5 µg/ml.

4) Viscosimetric Determinations.—Determinations were made before and after DNA injection on 2 ml of citrated plasma in a Couette viscosimeter, Lecomte de Nouy type (17). Results are given as specific viscosity (η sp) and reduced viscosity (η red), normal plasma being used as solvent.

This apparatus allows very sensitive measurements of viscosity at a low rate of shear (15 sec⁻¹). A variation of η sp from 0 to 0.05, roughly corresponding to 5–10 µg/ml of native DNA, is readily detected.

As a first approximation, [η] is proportional to the M.W. of DNA when degraded by DNase. As shown by Thomas (25)

\[ [\eta] = K'M \]  

(A)

On the other hand, η red is related to [η] by the Huggins equation

\[ \eta_{\text{red}} = [\eta] + k[\eta]^2c \]  

(B)

where k is a constant for a given DNA.

If, k, M.W., and [η] of the DNA initially used are measured, these 2 equations permit the calculation of the M.W. of the DNA in the animal blood plasma from the measures of η red and c.

When DNA is degraded further—[η] < 2000—Equation A becomes: \[ \eta_{\text{red}} = [\eta] (11). \]

The initial slope K (decrease of η sp/min) of the curve η sp = f(t) determined in vivo allows, under these conditions, the estimation of the rate of depolymerization (V₀) of the DNA (11).

Although the animals received the same quantity of DNA, the initial η sp varied slightly from one animal to another. To compensate for this variation, V₀ is given as K/η sp₀.

RESULTS

1. Fate of DNA after i.v. Administration.—For each rabbit, the decline of insol-DNA in plasma was a linear function of time, and consequently the quantity of insol-DNA which disappeared per unit of time was constant and independent of the quantity of insol-DNA actually present at each time. The rate of disappearance of insol-DNA (V) expressed as µg of insol-DNA/min/ml appeared to be proportional to the quantity of DNA injected, i.e., to the initial concentration of DNA (Chart 1). Although these results are apparently contradictory, an indirect explanation for them can be offered (see below under “Discussion”). Chart 2, A, allows a comparison between the specific viscosity of plasma and the concentration of insol-DNA versus time; specific viscosity decreased about
50% in less than 3 min, whereas the insol-DNA concentration did not vary.

In mice, the rate of disappearance of insol-DNA was constant in the first minutes following i.v. injection of DNA, but slower later (Chart 3).

This rate is correlated with the neutral DNase activity of plasma, both in rabbit and in mice (initial rate) (Charts 4, 5). The correlation coefficients between the 2 variables, viz. 0.88 and 0.98, are highly significant. The regression line intersects the ordinate very near the origin in the case of mice; in the case of rabbits, the regression line reached the ordinate far from the origin. Extrapolation gives an appreciable rate of insol-DNA disappearance for no DNase activity. But when the rate of DNA depolymerization ($V_D$) is expressed versus DNase activity, in contrast to what is observed when the rate of insol-DNA disappearance is plotted, a straight line going through the origin is obtained (Chart 6). Sol-DNA was not found to appear before or after injection of DNA, or of endogenous insol-DNA in rabbit plasma, for any DNase activity, at least at the level of sensitivity of the method (5 μg/ml).

On the other hand, sol-DNA (15-20 μg/ml) was found in mouse plasma 10-20 min after injection of DNA for each strain of mice used.

Injection of trisodium citrate did not significantly modify the rate of insol-DNA disappearance. After injection of DNA-MG (Chart 2, B), the insol-DNA concentration did not change much during the first 10 min and the plasma specific viscosity decreased very slightly. Such experiments must necessarily be short owing to toxicity of MG in doses needed to prevent dissociation of the DNA complex.

2. Fate of DNA after i.p. administration.—Considerable amounts of insol-DNA appeared in mouse blood (Chart 7). The highest concentration of insol-DNA and sol-DNA occurred at the same time as the highest viscosity, i.e., about 1 hr after injection of DNA. At this time, the reduced viscosity of blood was 1100 c.g.s., corresponding to insol-DNA of about $1 \times 10^6$ M.W. Complex formation with MG markedly slowed the passage of deoxyribonucleic material from peritoneum to blood, and the highest concentration of insol-DNA was observed only after 3 hr.

DISCUSSION

The plasma DNases play a fundamental and probably exclusive role in the initial degradation of DNA. Three experimental observations support this conclusion.

a) During the 3 min after DNA injection in blood there is a great deal of difference between the rapid decrease of viscosity indicating a depolymerization of DNA and the constant level of insol-DNA in plasma (Chart 2). Such data exclude the hypothesis of a rapid and important escape of native DNA out of vascular spaces during this initial step and demonstrate that the only significant phenomenon is the breaking down of phosphodiester linkages of DNA; this can only be due to enzymatic attack since shear degradation in the capillaries is very likely inoperative owing to the relatively low DNA M.W. ($\approx 6 \times 10^6$).

b) There is a proportionality between the initial rate of depolymerization ($V_D$) and the plasma neutral DNase activity level (Chart 6), a proportionality which is also found with the disappearance of insol-DNA in mice (Chart 5), as well as with the urinary excretion after i.v. injection of DNA-$\beta$-aminoisobutyric acid (Baiba), a specific catabolite of DNA-thymine (26).

c) The disappearance of the DNA-MG complex is remarkably slow when compared with the disappearance of DNA alone. It is unlikely that the DNA-MG complex, having a shape and a size similar to DNA, would diffuse out of vascular spaces at a diminished rate on mechanical grounds only; the most satisfactory hypothesis to explain this phenomenon is the strong DNase inhibition by MG.

Depolymerized, but still acid-precipitable DNA, produced from the initial depolymerization of native DNA is also subject to DNase action, but in this case, such an enzymatic action is associated with a diffusion out of vascular spaces. This is proved (a) in the rabbit by the rate of disappearance of insol-DNA versus DNase activity (Chart 4) extrapolated to zero activity, where it is still important (about 0.7 μg/ml/min); (b) in mice by the passage, in reverse, from peritoneum to blood of insol-DNA.

The insol-DNA diffusion out of the blood space is slower in mice than in rabbits. This difference probably explains the absence of sol-DNA and the apparent linearity of the rate of insol-DNA disappearance in rabbit blood: diffusion of insol-DNA would be higher during its degradation and thus would balance the slowing down of enzymatic degradation resulting from a decrease in substrate concentration.

Like plasma DNases, DNases from tissues seem to be active in the catabolic fate of injected DNA since the appearance of insol-DNA in plasma after intraperitoneal
CHART 2. Comparative study of the specific viscosity and insol-DNA concentration versus time in rabbit plasma (DNase activity 0.11 KU). (A) after i.v. injection of 10 mg of DNA (Sample c); (B) after i.v. injection of the complex DNA-MG (Sample c). \( [\eta] \) red are, respectively, in each case: (A) 7200, 4600, 3500, 2200, 1400, and 250; (B) 9500, 10400, 10100, and 8900 (c.g.s.). In the case of free DNA (A): \([\eta]\) was 5000, 3500, 2800, 2200, 1400, and 250, respectively; and M.W. was \(6 \times 10^4, 4.7 \times 10^4, 3 \times 10^4, 2.6 \times 10^4, 1.7 \times 10^4, \) and \(2.7 \times 10^4\), respectively.

CHART 3. Disappearance of insol-DNA in the plasma of 3 isogenic strains of mice after i.v. injection of 200 \(\mu g\) of DNA (Sample c).
injection of the relatively DNase-insensitive DNA-MG complex is delayed.

The existence of DNase inhibitors (5, 15) deserves some comment. Such inhibitors are released from blood cells in the plasma when the blood is withdrawn (7), but in most cases they are too dilute to influence the enzymatic kinetics in the Kurnick in vitro experimental conditions. Nevertheless, in a few cases, their concentration could be high enough, in spite of the dilution, to slow the rate of DNase action; this gives the most likely explanation of the 3 points found off the regression line in Chart 6, since these points correspond to the 3 samples of lower DNase activity. Owing to the presence of such inhibitors, an extrapolation from an in vivo experiment to an in vitro one is difficult (27).

Supposing some analogy between the modalities of action of bacterial transforming DNA and viral or cell DNA's which would have specific biologic activity in an appropriate cellular system, one can estimate approximately the minimum M.W. necessary for a DNA to retain an eventual biologic potency when it diffuses in blood circulation and, consequently, evaluate the time during which such an action can be expressed.

One hundred breaks on the molecule reduce the M.W. from $6 \times 10^8$ to $4 \times 10^8$ (25) and, on the other hand, reduce the biologic activity to 0.1% of the initial value (6, 21). For a 0.10-KU DNase activity, about 3 min are needed to reach such a DNA M.W. in rabbit plasma after i.v. injection of DNA.

If it is admitted that the biologic activity is in close inverse relationship with the number of phosphodiester linkages broken per molecule, from that observation the time necessary to obtain any given decrease of such an activity can easily be calculated. For example, 1 min after DNA injection, 90% of any possible biologic activity would be lost. This conclusion confirms the data of Herriot (7), who found that a transforming DNA loses 90% of its activity in a 12-min contact with a DNase solution with an activity the same as that of human plasma, which is about 10 times less active than rabbit plasma.
On the other hand, our data (Chart 1) show that the rate of degradation, expressed in proportion to the total number of breaks occurring per ml and per min, is roughly proportional to the initial DNA concentration; this means that the number of breaks per molecule is, for any DNA concentration, constant for a given DNase activity, as could be deduced from the kinetic studies on DNase (14). Those observations validated our conclusions for a large range of DNA concentrations, at least up to about 500 \( \mu g/ml \); such concentrations obviously correspond to amounts of DNA well below those that can be injected into mammals.

Our results do not exclude the ability of DNA to express its biologic potentialities in mammals after injection; even a small percentage of the injected material contains enough DNA molecules of a M.W. of \( 6 \times 10^8 \) to transform or infect most of the animal's cells and a low but significant amount of high M.W. DNA which, because of the lack of accuracy of usual technics for measuring blood volume, might have been absorbed from the plasma before the 1st determination.

Finally, these results emphasize how crucial the choice of species is when one tries to reveal biologic properties of DNA, since plasma DNase activities vary in a large range (5 \( \times 10^{-9} \) KU for man up to 2 KU for cat) (20).

They also show that DNase, besides some other possible defenses, is an effective biochemical barrier to exogenous DNA in mammals against which normal immunologic processes by antibody induction are not operating. Attempts to break this barrier will be feasible either through protection of substrates by complex formation with dyes or proteins or through use of natural DNase inhibitors (5, 15).

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