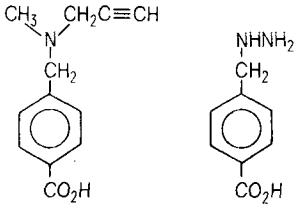
affinity chromatography in the dark and without prolonged contact with the column. For comparison, a benzylhydrazine inhibitor was also studied.

The required molecules for this work were the p-carboxy derivatives (**I** and **II**) of pargyline and of benzylhydrazine, which were prepared by nucleophilic displacement of bromine from p-carboxybenzyl bromide[19]. Heating excess N-methyl-N-propargylamine or hydrazine with p-carboxylbenzyl bromide gave, after concentration, extraction with benzene (**I** is soluble, while **II** is not), and conversion to the hydrochlorides with 3N HCl, 70% **I** · HCl, m.p. 209–210 °C (decomp.), or 70% **II** · HCl, m.p. 240 °C (decomp.).



(I) (II)

The inhibitors were coupled to AH-Sepharose 4B (Pharmacia, Inc.) using N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride and pH 8 Tris and pH 3 acetate buffers. A large excess of **II** was employed in the coupling step, as it undergoes self-condensation under the reaction conditions.

Pig liver MAO was prepared as described earlier[12] up to and including treatment with Triton X-100. The specific activity as defined by Tabor et al.[20] was seen to be 300. At this point, 2 ml of enzyme solution was added to the 1 × 5 cm affinity column containing pargyline, previously equilibrated with 50 mM phosphate buffer, pH 9.0. In view of the photochemical properties of the MAO inhibitor, all operations were performed in the dark at 4 °C. MAO adhered to the column which was further washed with 50 ml of 50 mM pH 9.0 phosphate buffer. The enzyme was then eluted from the column with either a salt gradient (0–1.0 M in NaCl) in pH 6.0 50 mM phosphate buffer or by the direct application of a 50 mM phosphate buffer (pH 6.0) 0.50 M in NaCl (figure). As is shown therein, the majority of the activity was eluted from the column as a single protein peak. The specific activity of this latter MAO peak was 3000 using 50 mM glycine buffer, pH 9.0, and benzylamine as the substrate[12] at 30 °C. This specific activity of the MAO obtained by a single affinity column treatment was thus identical to that of MAO obtained by the previously described method[13] involving two recrystallizations and 2 column treatments. Acrylamide gel electrophoresis (4%) showed a single dense band at pH 9.0. The affinity column is reusable if kept in a cool, dark environment and the enzyme purification process has been repeated 6 times with the same column.

All attempts to elute pig liver monoamine oxidase from an affinity column containing benzylhydrazine were without success. The enzyme readily attached itself to the column and resisted elution at high and low pH as well as with varying buffer and salt gradients.

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The role of sialic acid in determining the survival of circulating interferon

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Summary. Rabbit interferon has been extensively desialylated and its metabolic behaviour has been evaluated in the rabbit. The half-life of asialointerferon is significantly shorter than the native interferon and its urinary excretion becomes negligible. Moreover the rapid uptake of asialointerferon by the isolated and perfused rabbit liver, suggests a hitherto unsuspected catabolic pathway for this glycoprotein.

A striking feature of interferon (IF), either actively induced or passively administered, is its rapid disappearance from plasma[1,2]. While therapeutically this may appear somewhat disadvantageous[3], it reveals a deeper significance if it is considered that one of the functions of IF is to unleash the synthesis of the antiviral protein[4], and that a long-lasting plateau of IF in the body fluids could exert a depression of the immune response[5] or other deleterious effects[6]. It would then appear that catabolic or excretory systems have developed in order to remove rapidly the circulating IF as soon as this has been able, in some degree, to derepress the synthesis of the antiviral protein by interacting with the cell receptor[7,8].

In the past we and others have evaluated the possible catabolic role of the kidney[9], of the intestinal tract[9], of liver[10] and of body fluids[11], but nonetheless the main catabolic pathway has remained elusive.

The concept developed by Ashwell and Morell[12] that the terminal NAN has a role in regulating the survival time of glycoproteins in the circulation, and the fact that IF is a glycoprotein containing sialic acid (NAN)[13–17], may contribute a new approach to the study of the catabolism of this protein. Infact once NAN is cleaved by neuraminidase, galactose becomes exposed as the terminal sugar residue and acts as a specific determinant for recognition of the asialoglycoprotein by the hepatic receptor[18,19]. Thus, if desialylated IF is recognized and bound to the liver, its plasma disappearance should be considerably faster than that of native IF.

The problem has been approached by studying the behaviour in vivo and in vitro of native and desialylated rabbit IF and the results indicate that desialylation may represent an important first step in the catabolism of IF. Rabbit urinary and serum IF are obtained as previously described[9], but urinary IF has now been purified by

Table 1. The plasmatic clearance of homologous urinary interferon injected into rabbits

Native			Desialylated		
t $^1/_2$ (sec)	% of dose remained in the circulation after 15 min	% of dose excreted in the urine within 30 min	t $^1/_2$ (sec)	% of dose remained in the circulation after 15 min	% of dose excreted in the urine within 30 min
160 ± 10 (1)	15 ± 0,5 (2)	4.2 ± 1 (3)	92 ± 6 (4)	6 ± 0.9 (5)	0.3 ± 0.1 (6)

p (1:4) < 0.01, p (2:5) < 0.005, p (3:6) < 0.02.

extensive ultrafiltration using a 76 mm PM-30 Amicon Diaflo membrane fitting a 401 model. While the yield of the purified IF is practically unchanged, the specific activity increases about 12fold up to 8750 units/mg proteins. It has to be mentioned that urinary and serum IF are in fact urinary and serum proteins containing IF, and moreover urinary IF has the same physicochemical and biological properties as serum IF from which it is derived[9]. Interferon was titrated in serially propagated cell cultures RK13 by measuring viral inhibitory effect by plaque reduction method of VSV[9].

Desialylation of serum and urinary IF was carried out using neuraminidase of V. cholerae (Behringwerke) as previously described[14,15]. Incubation of 3 h caused the release of about 80% of NAN from urinary and serum IF with a small loss of antiviral activity. The obvious impossibility to assess the actual desialylation of IF in comparison to the bulk of contaminant glycoproteins, should not be a critical disadvantage because, as shown by Van den Hamer et al.[20] removal of as little as 20% of NAN from ceruloplasmin is enough to promote the binding of the partially desialylated protein.

Before reporting the present results, it must be mentioned that experiments carried out in different mammals[21–23] have all shown a very fast disappearance of IF from the plasma with half-lives of about 9 and 11 min. We anticipate that values of half-lives reported here are considerably shorter, and we proved that this is due to the fact that we measure the plasma volume for each rabbit, so that, by accurately knowing the plasma volume and the dose of IF injected, we can estimate exactly the 100% value at the time of injection. In any system with very high turnover of the component under study, this is the only correct method to assess the true 100% point at zero time. Although in laboratory animals blood mixing is fast, the first reliable experimental point can be withdrawn only 2.5–3 min after the injection, but, by this time, a considerable amount of the injected IF (and more if it is desialylated) has already disappeared. Therefore if the first experimental point is arbitrarily considered as the 100% value, the disappearance rate will be seriously underestimated. We have thought it worth while to emphasize this important methodological aspect because, in our opinion, it has been largely overlooked.

After injecting simultaneously the dose of IF with Evans Blue (0.6 mg), plasma volume was calculated[24] by extrapolation to zero time, from the samples obtained during the period 3–18 min after injection.

As an example the half-life of desialylated IF injected intravenously into a rabbit is, according to our procedure, equal to only 100 sec, whereas an artificially longer half-life (230 sec) is calculated if the first sample, withdrawn as early as 130 sec after injection, is taken as the 100% value. Indeed, by this time, 59% of the dose has already disappeared from the plasma.

After intravenous injection of native or desialylated homologous IF into rabbits, the half-life, the percentage of dose still present in the plasma 15 min after and the

Table 2. The uptake of homologous interferon by the isolated and perfused rabbit liver.

Sample	Native	Desialylated
Serum interferon	61	15
Urinary interferon	77 ± 1.4	25.7 ± 1.5

p < 0.001.
Values indicate the percentage of dose remained in the perfusate after 15 min.

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percentage of IF excreted into the urine within 30 min (after emptying and washing the bladder) were measured and results are reported in table 1 as mean ± S.E. The statistical significance of the difference was evaluated according to Student's t-test.

It appears that IF, once desialylated, remains in the circulation for a very short time and infact its half-life is almost halved. Moreover, it is interesting to note that only a negligible amount has been excreted with the urine, suggesting that the liver uptake may have increased.

For this reason the role of the liver has been tested by using the isolated perfused rabbit liver preparation[25]. The viability of the liver has been greatly improved in comparison to our previous study[10], by using an efficient oxygenator and fresh rabbit blood as perfusion medium. As shown in table 2, only 23–39% of native IF disappears in 15 min from the recirculating perfusate while the loss of desialylated IF is up to 74–85%. This striking difference suggests that the faster disappearance of desialylated IF from the perfusate is attributable to the binding of the asialointerferon to the liver.

Both the results in vivo and in vitro strongly suggest that the mechanism of IF catabolism is equivalent to that reported for several circulating glycoproteins[26]. Desialylation of IF is the preliminary essential step[27] and might occur in microenvironments with sluggish circulation or more likely by membrane-bound sialidase while IF is attached to the cell membrane[28].

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Isoenzymes of creatine phosphokinase in white blood cells*

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Summary. Creatine phosphokinase activity was found in human lymphocytes. Only MM type of the enzyme was detected in lymphocytes and polymorphonuclear leukocytes.

Creatine phosphokinase (CPK) plays an important physiological role in the energy metabolism of skeletal and cardiac muscle, and nerve cells. It catalyzes the reaction: creatine phosphate + ADP = ATP + creatine[2]. CPK exists in multiple forms. It is a dimer, composed of 2 types of subunits, designated M and B. These combine to produce three isozymes, designated MM, MB and BB[3].

MM type is mainly found in skeletal muscle, BB type predominantly in the nervous system and MB type in cardiac muscle[3]. Similarities between contractile muscle and mobile cells are widely recognized. Huxley[4] has recently pointed out that actin- and myosin-like proteins are present in motile non-muscular cells, such as amoeba, acanthamoeba castellanii, and blood platelets. Traniello et al.[5] has examined human polymorphonuclear leukocytes (PMNL) and found that these cells contain cytoplasmic CPK. However, no mention was made as to the isozyme of CPK present in the leukocytes. Meltzer and Guschwan have previously reported BB-type CPK in the platelets of rats and rabbits, although CPK activity was undetectable in human platelets[6]. We therefore investigated which type of CPK was present in lymphocytes and leukocytes. We wish to report that CPK activity is also present in human lymphocytes and that only MM type of CPK is present in lymphocytes and leukocytes.

Materials and methods. Leukocytes and lymphocytes were isolated by a modification of the methods described by Mendelsohn et al.[7] and Boyum[8]. 20 ml of venous blood were drawn from each of 4 normal subjects in heparinized non-sterile Vacutainers. With the Vacutainers oriented at a 45 °C angle, blood samples were allowed to stand for 1 h at 37 °C.

The resulting upper layer of leukocyte-rich plasma was collected and diluted to twice volume with Ca-Mg-free (CMF) Tyrode's solution, pH 7.4. 20 ml, at most, of this diluted plasma were layered over 9 ml of the following solution in a 50 ml conical plastic centrifuge tube (Falcon): 1.8 ml of 50% sodium diatriazoate (Hypaque, Winthrop Labs, New York, New York), 6.35 ml of 9% aqueous Ficoll (molecular weight, approximately 400,000, Sigma Chemical Co., St. Louis, Mo.) and 0.85 ml of water. Centrifugation was performed at 4 °C at 885 g for 15 min. The uppermost plasma layer was removed with a Pasteur pipette. The white fluffy lymphocyte-rich interphase layer originally between the plasma layer and the Ficoll solution was then collected by aspiration in 15 ml conical, plastic centrifuge tubes. Next, the remaining Ficoll layer was carefully removed, leaving a leukocyte-rich layer at the bottom of the tube. This leukocyte-rich layer was resuspended in 2 ml of CMF and collected by aspiration. Each lymphocyte-rich fraction and each leukocyte-rich fraction were washed twice by resuspension in 10 ml CMF and centrifugation at 110 × g for 10 min in order to remove platelets, plasma and Ficoll. After washing, all lymphocyte-rich fractions were combined in one 15 ml

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