The results indicate that the magnitude of 4-^{14}C-cholesterol incorporation roughly paralleled the pattern of chemical composition of cholesterol esters in different species.

The rates of overall esterification of serum cholesterol, i.e. the sum of all cholesterol esters in vitro in examined species, exhibited great variations; nevertheless no significant differences of the rate of esterification between the species studied have been found.

The experiments reported here illustrate that cholesterol esters formed during the in vitro incorporation with 4-^{14}C-cholesterol resembled roughly the preexisting pattern of serum cholesterol esters in all 4 species studied. Thus in guinea-pig, with highest relative magnitudes of esterification of diunsaturated esters, the level of these esters was also highest. This is corroborated by the data obtained by others by means of gas-chromatography where also about 58–66% of diunsaturated cholesterol esters in guinea-pig's serum have been reported[8,9]. The percentage of esterification in the fraction of diunsaturated esters was somewhat lower in man and rabbit; in fact, in this respect, man is quite similar to the rabbit. On the other hand, the rat displays a different pattern of serum cholesterol esters formation with predominating tetra- and triunsaturated esters. These findings are in agreement with GOODMAN's statement[1] concerning the problem of the applicability to man of studies on cholesterol ester metabolism conducted in the rat.

The similarity between the relative magnitudes of esterification and cholesterol esters composition in serum of the species studied indicate that esterification of cholesterol in the blood per se (presumably by means of cholesterol acyltransferase) might be a major source of plasma cholesterol esters. This is corroborated by the findings of predominating hydrolysis of cholesterolesters in the liver[10], possibly by the net decrease of cholesterol esters in the liver, contrary to the serum, during in vitro incubation[11].

The data presented here indicate that the rate of total cholesterol esters formation in vitro in man, guinea-pig, rat and rabbit did not substantially differ. It therefore seems improbable that the reaction of total cholesterol esterification in the blood is in any relation to differing species susceptibility to atherosclerosis[12].

Zusammenfassung. Die Bildung von Cholesterinester wurde in vitro im Serum verschiedener Species geprüft.

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Isoelectric Fractionation of Desialyzed Interferon

We have previously reported[1] that, if sialic acid is a constituent of interferon, it has no role in its activity. This conclusion does not rule out the presence of this sugar in the interferon and we anticipated that it would be worthwhile to evaluate the electrophoretic behaviour of interferon treated with neuraminidase. Assuming that interferon contains sialic acid, and that the carbohydrate can be extensively cleaved by the enzyme, the isoelectric point should rise and the electrophoretic mobility should consequently be reduced. This reasoning has been found correct in the case of haptoglobin, transferrin[2] and serum cholinesterase[3].

Our previous studies[4,5] on the electrophoretic behaviour of interferon in different supporting media have shown a very broad range of mobility and have suggested that interferon activity is due to molecules differing not only in size but also in electric charge. This may be partly contributed by the carboxyl group of sialic acid which, if in a different amount, could enhance the charge, hence the mobility.

The isoelectric focusing in polyacrylamide gel of neuraminidase-treated interferon was the technique used and the technical details and results are as follows: Urinary interferon was obtained from male rabbits after i.v. inoculation of Newcastle disease virus (NDV) as previously described[6]. 100 ml of dialyzed urine yielded aboud 90 mg of proteins that were lyophilized and stored under refrigeration.

Interferon was titrated in baby rabbit kidney cell cultures by measuring viral inhibitory effect by plaque reduction method of vesicular stomatitis virus (VSV)[5]. The average specific activity of urinary interferon was 5000 U/mg urinary proteins.

Neuraminidase of *V. cholerae* was obtained from Behringwerke and the enzyme preparation was reported to be protease-free and contained 500 U/ml.

Urinary proteins containing interferon (10 mg/ml) dissolved in 0.15 *M* NaCl, 0.05 *M* sodium acetate-acetic acid buffer (pH 5.5) and 20 m*M* $CaCl_2$ were incubated at 37°C in the presence of chloroform for 4 h with a total of 1000 U of neuraminidase (the second lot of 500 U was added after 2 h of incubation). Control samples were incubated without the enzyme in the same conditions. At the end of incubation the samples were icecooled and, after taking suitable aliquots for measuring free and bound sialic acid, were dialyzed against 0.3 *M* sucrose.

Sialic acid content was measured according to AMINOFF[7]. Total sialic acid content was measured from the original sample hydrolyzed in 0.05 *N* H_2SO_4 at 80°C for 1 h. Residual sialic acid content and interferon activity are expressed as a percentage of the original at the beginning of incubation.

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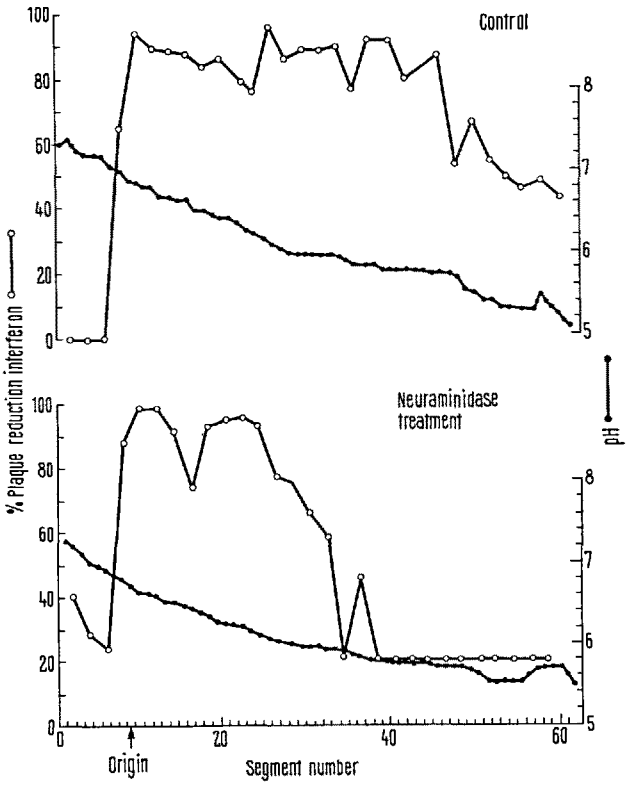
By using a short incubation period, it has been possible to obtain almost complete desialization while preserving 94% of the original interferon activity.

Polyacrylamide gel was prepared by the polymerization of 6% (w/v) acrylamide containing 2% (w/v) 'Ampholine' carrier ampholytes (pH 5–8). Polymerization of the gel was induced by riboflavine. After loading the samples (neuraminidase-treated and control urinary proteins containing interferon), the surface of the gel was covered with petroleum jelly and isoelectric-focusing[8] was carried out in the vertical position: the cathode was wetted with 5% (v/v) ethylenediamine solution and the anode with 5% (v/v) phosphoric acid. A dialysis membrane prevented liquid flow from the electrode vessels to the gel. The run was carried out in the cold room for 30 h with a potential difference of 9 V/cm.

At the end of the run, the gel was divided[9] into 61 segments (0.3 cm) for the neuraminidase-treated and control samples, respectively. In order to measure the pH, $^1/_3$ of the segment was suspended in 4.5 ml of distilled water; in order to recover the fractionated interferon, the remaining segment was immersed into 5 ml of medium 199 containing 20% bovine serum and was left shaking for 60 h at 0°.



Simultaneous separation of neuraminidase-treated and control urinary proteins containing interferon by polyacrylamide – isoelectric – focusing in a pH 5–8 ampholyte system.

The Figure shows the result of a typical run: the control urinary interferon consists of several active components (maybe 7 or more) of different isoelectric point ranging from 6.9 to 5.5. After desialyzation the electrophoretic pattern is strikingly changed and shows 2 main peaks having isoelectric points at pH 6.6 and 6.3 while most of the more acidic interferon has disappeared. The heterogeneity of the interferon seems therefore due, at least in part, to the presence, possibly in a different amount, of sialic acid in the molecule.

The result suggests strongly that interferon is a glycoprotein and that removal of sialic acid raises the isoelectric point and reduces the electrophoretic mobility. The reason(s) of interferon heterogeneity remains as yet conjectural; in fact it could be due to difference in sialic acid addition at the site of synthesis, as it could be due to partial desialyzation during transit in the body or during purification procedures.

While this manuscript was in preparation a report by SCHONNE et al.[10] on isoelectric focusing of rabbit interferon has appeared. Although the source of interferon was different, the same conclusion has been reached in both studies[11].

Riassunto. Proteine urinarie di coniglio contenenti interferone sono state desializzate mediante neuraminidasi e sono state separate simultaneamente ai controlli mediante elettroforesi su poliacrilammide con gradiente di pH. La rimozione dell'acido sialico produce una notevole modificazione del profilo elettroforetico con diminuzione dell'attività interferonica avente punti isoelettrici inferiori a pH 6.3. Il risultato indica che almeno in parte l'interferone contiene acido sialico.

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Experimental Lathyrism: Inhibition of β-Alanine Incorporation by β-Aminopropionitrile

The nature of the defect in experimental lathyrism has been extensively reviewed by other investigators[1–3]. Essentially, it is a disease of connective tissues exhibiting changes in collagen, elastin, and ground substance.

The nature of the defect in collagen has been shown to be an increase in the amount of the neutral salt soluble fraction, and the failure of collagen molecules to mature into normal fibrils. This defect has been attributed to modification in the cross-linking between collagen molecules occurring at both the inter and intramolecular level[4,5]. Changes in elastin are also thought to be due to a failure in cross-linking. Whether this is accompanied by a decreased synthesis of elastic fibers or a destruction and fragmentation of those fibers already produced is not clear[6,7]. Changes in the ground substance have been attributed principally to alterations in mucopolysaccharides.