dinioacetic and guanidinosuccinic acid) and from ammonia, which interferes with the color formation. A high sensitivity (lower limit: 0.005 mg/100 ml) is obtained by performing the Sakaguchi reaction on a low volume of fluid containing all the MG eluted quantitatively from the resin.

Method. Dowex resin (50-W 12, 100–200 mesch) is converted to the Na form by running 50 ml of 0.1 *N* NaOH through the column (6 × 20 mm). 20 ml for normal serum, 10 ml for uremic serum and normal urine and 2–5 ml for urine from uremic patients are made up to 100 ml with 0.01 *N* NaOH and run through the column, followed by 100 ml of 0.1 *N* NaOH and by 50 ml of deionized water. After this is passed completely, 50 ml of 2 *N* HCl are run, collected in a 200 ml beaker, and dried at 70°–80 °C in a ventilated oven. The dry residue is dissolved in 10 ml of 0.1 *N* NaOH which are filtered through paper and the Sakaguchi reaction (according to YATZIDIS et al.[1]) is performed on 8 ml of the clear filtrate. Color is read at 500 nm against a blank consisting of 8 ml of 0.1 *N* NaOH containing 15 g/l of NaCl. The standard curve is made by treating 10 ml aliquots of dilutions from 0.005 to 0.500 mg/100 ml of MG (as base) exactly like biological fluids.

Results. The percent recoveries of MG added (in amounts from 0.5 to 50 μg) to 10 ml of serum and urine from normal and uremic subjects, ranged from 85 to 102 (mean 93). In 15 samples of normal human serum the average MG concentration was 0.008 ± 0.002 mg/100 ml and its daily urinary output in 8 healthy adults on a freee diet (2 measurements each in consecutive days) was 1.83 ± 0.29 mg (concentrations ranged from 0.07 to 0.20 mg/100 ml).

In 82 acute and chronic renal patients, serum concentrations of MG ranged from normal values in the slightest cases (plasma CR concentrations below 2 mg/100 ml), to a maximum value of 0.60 mg/100 ml in an anuric patient (polycistic kidney disease) with a plasma CR content of 26 mg/100 ml. A direct correlation was found between the serum levels of CR and those of MG, but the ratio: serum MG/serum CR was significantly higher in the anuric patients. The daily urinary excretion of MG was found to be elevated in patients with renal failure (68 determinations in 35 cases) reaching values as high as 30 mg/24 h in the most severe ones (concentrations raeched levels of 3.0 mg/100 ml).

Discussion. Paper chromatography and electrophoresis performed on the dry residue of the HCl eluate dissolved in ethanol, revealed the only Sakaguchi-positive spot of MG thus showing that the procedure described is specific for it.

The elution of MG from the resin column with 60 ml of 4 *N* KOH, according to Carr et al.[5], and the reading of the color developed in it on a suitable standard curve, yielded results not significantly different from those obtained with the procedure described, provided the MG concentrations in the body fluid samples were higher than 0.20 mg/100 ml. This alkaline elution is not an advantageous alternative to the acid one, for it reduces the sensitivity and the accuracy of the method; however, the equality of the results obtained with the two procedures permits us to exclude that unknown substances (other than creatine and CR which are quantitatively removed from the resin with the preliminary elution with 0.1 *N* NaOH) are converted to MG during drying of the HCl eluate.

It can thus be concluded that MG is present in normal serum and urine and that it is retained in humans suffering for renal failure, as it was previously found to occur in nephrectomized dogs[5]. The reason for which high amounts of MG are excreted by uremics, as previously found by STEIN[6], is unclear at present.

Normal dogs severely intoxicated with MG suffer from symptoms resembling those of uremia[7], and our unpublished observations demonstrate that equal though milder symptoms appear even when their plasma MG levels are maintained as high as those found in severely uremic patients[8].

Riassunto. Viene descritto un metodo di dosaggio della metilguanidina nel siero e nell'urina e vengono riportati i valori normali della concentrazione serica (0.008 ± 0.002 mg/100 ml) e della eliminazione urinaria (1.83 ± 0.29 mg/24 h). Tanto la concentrazione serica quanto la eliminazione urinaria degli uremici sono risultate elevate.

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The Incorporation of 4-^{14}C-Cholesterol into Different Cholesterol Esters of the Blood Serum in Man, Guinea-Pig, Rat and Rabbit

Factors influencing the level of cholesterol esters in blood are at present not fully defined. Nevertheless there is increasing evidence about the importance of the esterification of cholesterol in the blood plasma per se (see the comprehensive survey by GOODMAN[1]).

At present it is generally accepted that the enzyme responsible for the esterification of cholesterol in the plasma of man, rat and rabbit is lecithin-cholesterol acyltransferase[2–4]. There appears to be some uncertainty whether the pattern of newly formed labelled cholesterol esters obtained after the incubation of a given plasma with radioactive cholesterol will resemble that existing normally in that plasma[4]. Therefore the experiments were carried out to investigate the cholesterol esterification in the serum of four species with different pattern of the cholesterol esters.

Fresh serum, obtained after centrifugation of blood which was allowed to clot at room temperature, was always used. 4-^{14}C-cholesterol (Radiochemical Centre, Amersham) adsorbed to celite has been added to the

Esterification of cholesterol in the blood serum of man, guinea-pig, rat and rabbit

Group	No. of individuals		Cholesterol esters					
			saturated	1=	2=	3,4=	x=	Σ
Man	4	µg/ml/h[a]	0.93 ± 0.03	1.75 ± 0.03	2.77 ± 0.58	0.46 ± 0.01	0.13 ± 0.03	6.04 ± 0.85
		%	15.4	29.0	45.9	7.6	2.1	
		mg/ml[b]	0.26	0.42	0.49	0.14	0.07	1.38
		%	18.8	30.4	35.6	10.1	5.1	
		S.A.[c]	1.07	1.28	1.73	1.00	0.57	5.65
		%	18.9	22.7	30.6	17.7	10.1	
	5	µg/ml/h	0.90 ± 0.22	1.56 ± 0.40	2.78 ± 0.56	0.64 ± 0.22	0.06 ± 0.01	5.94 ± 1.02
		%	15.2	26.3	46.8	10.7	1.0	
		mg/ml	0.24	0.42	0.57	0.15	0.03	1.41
		%	17.0	30.0	40.2	10.5	2.1	
		S.A.	0.58	0.57	0.75	0.67	0.33	2.90
		%	20.0	19.7	25.9	23.1	11.3	
Guinea-pig	5	µg/ml/h	1.02 ± 0.07	1.08 ± 0.18	4.26 ± 0.98	0.81 ± 0.45	0.12 ± 0.04	7.29 ± 1.58
		%	14.0	14.8	58.4	11.1	1.7	
		mg/ml	0.06	0.06	0.18	0.04	0.01	0.35
		%	17.1	17.1	51.4	11.4	2.9	
		S.A.	5.83	6.16	8.22	7.0	4.0	31.21
		%	18.7	19.7	26.4	22.4	12.8	
Rat	3	µg/ml/h	0.46 ± 0.02	0.66 ± 0.10	1.61 ± 0.20	2.75 ± 0.40	0.38 ± 0.10	5.86 ± 1.01
		%	7.8	11.3	27.5	47.0	6.4	
		mg/ml	0.08	0.08	0.10	0.09	0.04	0.39
		%	20.5	20.5	25.6	23.1	10.3	
		S.A.	4.93	6.41	13.16	23.65	7.20	55.35
		%	8.9	11.6	23.8	42.8	13.0	
	4	µg/ml/h	0.65 ± 0.11	0.88 ± 0.10	2.30 ± 0.39	5.29 ± 0.94	0.73 ± 0.18	9.85 ± 1.11
		%	6.6	8.9	23.4	53.7	7.4	
		mg/ml	0.08	0.12	0.17	0.20	0.13	0.70
		%	11.4	17.1	24.3	28.6	18.6	
		S.A.	6.75	6.16	11.29	22.0	4.69	50.89
		%	13.3	12.1	22.2	43.2	9.2	
Rabbit	3	µg/ml/h	0.62 ± 0.11	0.94 ± 0.02	2.13 ± 0.20	0.38 ± 0.01	0.11 ± 0.01	4.18 ± 0.80
		%	14.8	22.5	51.0	9.1	2.6	
		mg/ml	0.08	0.07	0.11	0.05	0.03	0.49
		%	23.5	20.6	32.4	14.7	8.8	
		S.A.	5.25	9.00	14.00	5.20	2.66	36.11
		%	14.5	24.9	38.8	14.4	7.4	
	5	µg/ml/h	0.93 ± 0.22	1.50 ± 0.49	2.50 ± 0.27	0.40 ± 0.03	0.04 ± 0.01	5.37 ± 0.95
		%	17.3	27.9	46.6	7.5	0.7	
		mg/ml	0.17	0.19	0.20	0.08	0.06	0.70
		%	29.8	32.6	35.1	1.4	1.1	
		S.A.	5.17	7.68	11.90	4.75	0.66	30.16
		%	17.1	25.5	39.5	15.7	2.2	

Values are mean ± S.D. 1=, monounsaturated cholesterol esters; 2=, diunsaturated cholesterol esters; 3,4=, tri- and tetraunsaturated cholesterol esters; x=, polyunsaturated cholesterol esters. [a] µg of cholesterol esterified/h/ml serum. [b] mg of cholesterol/ml serum, pooled sample. [c] % of the total activity in the tube/µg of cholesterol.

serum, essentially according to AVIGAN's method[5]. The incubation was performed under steady agitation for 3 h at 37 °C in a metabolic incubator.

After incubation the lipids were extracted and separation of cholesterol esters into saturated, mono-, di-, tri- and tetra- and furthermore polyunsaturated esters was achieved according to previously described methods[6,7], using TLC on silica gel. For chemical determination[8] the pooled sample of all specimens in the given series has been used. The radioactivity was determined on Nuclear Chicago Liguid Scintillation Computer Mark I.

The blood has been collected from female Wistar rats weighing 160–200 g and guinea-pigs (ca. 350 g) after decapitation, from Chinchilla female rabbits (ca. 2.5 kg) from ear vein, and in normal female persons (aged 20–45 years) from cubital vein. Concerning the metabolic state the blood was withdrawn after overnight fasting in human beings, while in animals food and water was supplied ad libitum. Number of examined persons and animals is given in the Table.

From the Table it is apparent that the formation of cholesterolesters as well as the specific activity were greatest in the fraction of diunsaturated cholesterol esters in man, guinea-pig and rabbit. The different pattern was observed in the rat where the esterification in the fraction of tri- and tetraunsaturated cholesterol esters has been predominating.

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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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