protein might result in different effects on the vascular endothelium.

From the data shown in Tables I and II, it would appear that aortic atherosclerosis of the rabbits was more severe when the plasma protein levels were increased. The injections did not increase the protein levels significantly, though. Electrophoretic determinations showed that the protein levels were merely raised to the upper end of the normal range for rabbits. Thus, it may be possible that such changes could occur in the normal, ageing, human population.

In addition, it would appear that small dosages of foreign proteins (e.g., the human γ-globulin in this case) cause vascular damage similar to that caused by the species-specific protein. The fact that foreign proteins have been seen to cause changes in the permeability may thus, in fact, be partially due to their effect on the oxygen transport rate, instead of totally due, as is frequently assumed, to immunological reactions.

Résumé. Les examens macroscopiques et microscopiques effectués sur l'aorte des lapins ont été mis en corrélation avec le degré de concentration de la protéine plasmique. Les résultats indiquent qu'une augmentation des protéines plasmiques correspond a un degré plus élevé d'athérosclérose.

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Determination of the Vitamin A Bodypool of Rats by an Isotopic Dilution Method

An established feature of the metabolism of vitamin A is its accumulation and storage in the liver[1]; the size of this vitamin A store, however, cannot be determined by measuring the plasma vitamin A level since this level is in a constant range as long as the liver stores of the vitamin are not exhausted[2].

On the basis that a dose of vitamin A is transported to the liver by the blood and then takes part in a dynamic vitamin A exchange between liver, blood and the vitamin A-requiring organs[3], it may be assumed that the portion of an administered dose of labelled vitamin A in the normally constant plasma vitamin A level is small if the preexistant vitamin A bodypool has been large, and will be higher if the bodypool of vitamin A has been low. In order to find out whether this assumption is correct and could be used as a basis for an assay of the vitamin A bodypool, the following experiments have been carried out.

Experiment 1 was designed in order to prove that a dose of labelled vitamin A will be mixed within a certain time with the preexisting vitamin A bodypool of the rat. Thus, it was to be expected that the specific radioactivity of the vitamin A of plasma and liver would become the same after the mixing period. Table I shows the relatively constant levels of vitamin A in the plasma of 6 different rats; the total radioactivity, however, and the specific radioactivity of the plasma vitamin A differ significantly, obviously in correlation with the vitamin A status of the animals. Assuming that the specific radioactivity of the plasma and the liver vitamin A were equilibrated in each animal, the liver vitamin A was calculated by dividing the total radioactivity of the liver by the specific radioactivity of the plasma vitamin A. The calculated numbers and the result of fluorometric determinations of the liver vitamin A are in good agreement at least for animals with a low bodypool of vitamin A. These data indicate that in fact a new dose of vitamin A seems to become homogeneously intermixed with the preexisting vitamin A pool of the liver.

In experiment 2, the labelled vitamin A was injected i.v. in order to establish the same liver levels of labelled vitamin A in all experimental animals. Table II shows that the plasma vitamin A is quite similar in the different rats; but, as in the first experiment, the significantly differing values for the absolute radioactivity in the plasma and the specific radioactivity of the plasma vitamin A indicate little dilution of the labelled vitamin A by a small

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Table I. Specific radioactivity of plasma vitamin A; calculated and fluorometrically determined vitamin A content of livers (Experiment 1)

Rats treated with 10 μC of 6,7-$^{14}C_2$-retinol	Plasma			Liver		
	IU vit. A (100 ml)	cpm (100 ml)	cpm/IU vit. A	cpm (kg liver)	Calculated IU vit. A (g liver)	Analyzed IU vit. A (g liver)
On normal diet	135	58,600	434	397×10^6	915	1,680
On normal diet	135	99,100	734	466×10^6	635	920
Vitamin A-deficient diet	135	2,540,000	18,814	205×10^6	11	15
Vitamin A-deficient diet	124	2,140,000	17,258	509×10^6	30	30
Vitamin E-deficient diet	92	516,000	5,608	348×10^6	62	50
Vitamin E-deficient diet	102	708,000	6,941	456×10^6	66	53

Rats received orally an oily solution of 10 μC of 6,7-$^{14}C_2$-retinol (specific radioactivity: 43 μC per mg) and were killed after 5 days. Vitamin A in plasma and liver was determined by direct fluorometric measurement in the fat extract as described elsewhere[7]. Radioactivity was determined by tissue combustion according to KALBERER and RUTSCHMANN[8].

Table II. Specific radioactivity of plasma vitamin A; calculated and measured vitamin A content of liver (Experiment 2)

Rats (female) treated with 81.2 μC of 11,12-$^3H_2$-vitamin A acetate	Plasma			Liver		
	IU vit. A (100 ml)	cpm (100 ml)	cpm/IU vit. A	Assumed storage of dose (%)	Calculated IU vit. A (g liver)	Analyzed IU vit. A (g liver)
4 rats on normal diet	119	753,600	6,333	80	647	471
	140	1,291,200	9,222	80	330	378
	124	635,000	5,122	80	678	612
	91	644,000	7,085	80	526	465
4 rats on vitamin E-deficient diet	102	2,793,600	27,388	50	151	145
	75	2,427,200	32,363	50	102	71
	86	3,367,200	39,153	50	77	57
	113	3,606,400	31,915	50	79	63

Rats were injected i.v. with an aqueous emulsion of labelled vitamin A. They were decapitated 11 days later. Determination of vitamin A and radioactivity in plasma and liver as in experiment 1. The calculation of the vitamin A content of the liver was based on the fact that 80 to 90% of the vitamin A content of the body is deposited in the liver[4].

vitamin A pool in the case of the vitamin E-deficient rats, and higher dilution by a normal vitamin A pool in the normal rats. For the calculation of the bodypool of vitamin A, a storage of 80% of the vitamin A dose was assumed in the normal rats and of 50% in the vitamin E-deficient animals, based on empirical figures[4]. Storage radioactivity divided by the specific radioactivity of the plasma vitamin A led to figures for the vitamin A bodypool which were comparable to those obtained by the fluorometric determination of the liver vitamin A.

In the previous 2 experiments, the animals were treated with fairly high doses of radioactivity in order to label sufficiently the plasma vitamin A; this high dose, however, also increased the vitamin A bodypool of the animals to an undesirable degree. In experiment 3, we therefore tried to find the lowest possible dose of radioactivity which would cause a just measurable isotope level in the blood of normal rats. The results in Table III indicate that a dose of 0.65 μC of tritiated vitamin A acetate per 100 g of animal weight was sufficient for labelling the plasma vitamin A for radioactivity measurements. The calculation of the vitamin A bodypool of the rats again yielded numbers which are comparable to the fluorometrically determined liver levels of vitamin A.

The results of the 3 experiments demonstrate that an oral or i.v. dose of labelled vitamin A is diluted by the preexisting vitamin A bodypool leading to equal specific radioactivities of the vitamin A in blood and liver a certain time after treatment. This is in agreement with the assumption of SEWELL et al.[3] that the total body vitamin A is in a dynamic state; a continuous turnover of the vitamin A bodypool is necessary for the equilibrium of its specific radioactivity in all the tissues. Recently, RAICA et al.[5] reported a 7-day period to be necessary for attaining this equilibrium in rats. On this base, it becomes possible to calculate the vitamin A content of liver from its total radioactivity and the equilibrated specific radioactivity of the plasma vitamin A after treatment with labelled vitamin A. In animals with a normal vitamin A supply, the half life time of vitamin A storage in the body by far exceeds the equilibration period; thus the vitamin A pool of the whole body of the rat can also be estimated simply from the absolute radioactivity of the administered dose and the resulting specific radioactivity of the plasma

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Table III. Specific radioactivity of plasma vitamin A; calculated and analyzed vitamin A content in liver (Experiment 3)

Rats (100 g, male) treated with	Plasma			Liver	
	IU vit. A (100 ml)	cpm (100 ml)	cpm/IU vit. A	Calculated[a] IU vit. A (g liver)	Analyzed IU vit. A (g liver)
65 μC ^{3}H-vit. A acetate	146	2,444,800	16,745	394	373
65 μC ^{3}H-vit. A acetate	178	4,060,800	22,813	297	334
6,5 μC ^{3}H-vit. A acetate	172	676,800	3,934	172	233
6,5 μC ^{3}H-vit. A acetate	129	506,933	3,929	181	250
0,65 μC ^{3}H-vit. A acetate	135	52,000	385	165	195
0,65 μC ^{3}H-vit. A acetate	183	60,000	327	190	219
0,065 μC ^{3}H-vit. A acetate		(11,200)			
0,065 μC ^{3}H-vit. A acetate		(3,200)			

[a] Based on the assumption of 80% storage of the dose in the body.

Rats were treated i.v. with an aqueous emulsion of 11,12-$^3H_2$-vitamin A acetate and were decapitated 5 days later. Vitamin A and radioactivity of plasma and liver were determined as in experiment 1.

vitamin A. Conditions for the applicability of this assay of the vitamin A bodypool to animal populations without further modifications are constant absorptions by the animal livers of the injected labelled vitamin A from the blood and constant long storage half life times for the vitamin A in the total body.

The observations of low vitamin A reserves in vitamin E-deficient rats, which is in agreement with the findings of several other laboratories[6], illustrates the usefulness of this assay. Further investigations are in progress.

Zusammenfassung. Es wird eine Isotopen-Verdünnungstechnik zur Berechnung des Vitamin-A-Körpervorrates in Ratten beschrieben. Aus der Verdünnung einer i.v. Dosis radioaktiven Vitamins A durch das unmarkierte Vitamin A des gesamten Körpers kann der Vitamin-A-Körpervorrat berechnet werden. Die so erhaltenen Werte sind mit denen der analytischen Vitamin-A-Bestimmungen in den entsprechenden Lebern vergleichbar.

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Synthesis of [4-Leucine]-Arginine-vasotocin, a Natriuretic Analogue of Arginine-Vasotocin

Oxytocin (I) and the vasopressins have been reported to be natriuretic in mammals under certain conditions[1]. The natriuretic properties are accentuated in the synthetic hormone analogues[2] [Leu^4]-oxytocin[3-5] (IIa), [Leu^4]-mesotocin[6,7] (= [Leu^4, Ile^8]-oxytocin, IIb), [Leu^3, Leu^4]-oxytocin[8] (IIc), [Leu^2, Leu^4]-[9] and [Ile^2, Ile^4]-oxytocin[5,10] (IIIb, c) and, to a lesser extent, [Ile^4]-oxytocin[5] (IIIa).

Since of all the natural neurohypophysial hormones it is arginine-vasotocin (= [Arg^8]-oxytocin, IVa), the endogenous hormone of the lower vertebrates, which has the greatest effect on the transport of sodium by amphibian membranes (skin, bladder) as well as the amphibian kidney[11] it appeared of interest to carry out a similar structural change – replacement of glutamine by leucine in sequence position 4 – also in the molecule of arginine-vasotocin and to examine the properties of the resulting analogue, [Leu^4]-arginine-vasotocin (= [Leu^4, Arg^8]-oxytocin or [Ile^3, Leu^4]-arginine-vasopressin, IVb).

Physical constants of new synthetic intermediates[a]

No.	Compound	M.p. (°C)[b] $[\alpha]_D^{25}$[c]
V	Z-Leu-Asn-Cys-Pro-Arg-Gly-NH_2 (Bzl, Tos)	183–185 / −41.6°
VI	Z-Ile-Leu-Asn-Cys-Pro-Arg-Gly-NH_2 (Bzl, Tos)	211–212 / −41.9°
VII	Z-Tyr-Ile-Leu-Asn-Cys-Pro-Arg-Gly-NH_2 (Bzl, Bzl, Tos)	237–238 / −36.2°
VIII	Z-Cys-Tyr-Ile-Leu-Asn-Cys-Pro-Arg-Gly-NH_2[d] (Bzl, Bzl, Tos)	220–223 / −42.6°
IXa	Z-Leu-Asn-Cys-OMe (Bzl)	195–197 / −28.0°
IXb	Z-Leu-Asn-Cys-N_2H_3 (Bzl)	230–234 / −38.2°

[a] Satisfactory elemental analyses were obtained for all the compounds listed. [b] Melting-points were determined on a microscope hot-stage. [c] In dimethylformamide, c = 0.5 (V–VIII) or 1.0 (IXa, b). [d] Monohydrate.

The protected nonapeptide VIII (Table) was prepared from the known[12] carboxyl-terminal pentapeptide derivative, Z-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2, by a stepwise procedure using benzyloxycarbonyl-protected *p*-nitrophenyl esters for addition of the individual amino-acid residues and hydrogen bromide in acetic acid for cleavage of the benzyloxycarbonyl protecting groups[13]; the intermediates are listed in the Table. In an alternative synthesis, VIII was obtained from 3 tripeptide units: The hexapeptide V was prepared from Pro-Arg(Tos)-Gly-NH_2[14] and the azide obtained from the tripeptide hydrazide IXb (made in turn from the ester IXa) and, after removal of the benzyloxycarbonyl group, condensed with the azide prepared from Z-Cys(Bzl)-Tyr-Ile-N_2H_3[15].

Removal of the protecting groups from VIII and formation of the disulphide bridge by standard procedures[16]

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