lack clear antithrombotic activity, can nevertheless inhibit ADP-induced aggregation in vitro; among these compounds β-blocking drugs are of particular interest[4,5]. We have carried out trials to estimate the inhibitory effect of some β-blocking drugs and to check whether a dose - response relationship was present.

Materials and methods. ADP-induced platelet aggregation, in human PRP (about 3×10^5 plat/ml), was measured by the turbidimetric method of BORN and CROSS[6] using an E.E.L. Long Cell Aggregometer. All trials were carried out with 1.2 ml as follows: 0.8 ml of PRP, 0.2 ml of Michaelis buffer (or β-blocking drug) and 0.2 ml of ADP. ADP concentration was calculated separately for each trial and a dose of 2K was used, where K is the dose of ADP which induced 50% of the maximum aggregation. Details of the method will be reported elsewhere[7]. The following agents were used: ADP and four β-blocking drugs (propranolol, pindolol, INPEA and 1-isopropylamino-3-(1, 2, 3, 4-tetrahydro-1, 4-ethanol-5-naphtoxy)-2-propanol H Cl, or K 4423[8,9].

Results and discussions. The inhibitory effect of the β-blocking drugs was proportional to the doses used. Analysis showed a highly significant dose-effect regression. In view of this finding we extrapolated from each function the ED_{50}, i.e., the dose of the drug capable of reducing the effect of 2K-ADP to that of 1K-ADP. Analysis of variance on the ED_{50} values showed significant differences between the reference drug (propranolol) and the other compounds. From the data reported in the Table, pindolol proved more active than K 4423, which in turn was more active than propranolol and INPEA.

Mean values and s.e. of equipotent doses (μg) of β-blocking drugs on ADP-induced human platelet aggregation

	Propranolol	Pindolol	INPEA	K 4423
Mean	21.12	6.35	305.63	13.66
s.l.	2.92	1.60	54.27	3.21
Replications	27	12	5	10

These results are in good agreement with data for the same drugs obtained from pharmacological tests on isolated organs[10,11] considered specific for the assessment of activity of β-blocking drugs[12].

Riassunto. L'effeto inibente di farmaci β-bloccanti sull'aggregazione di piastrine umane indotta in vitro mediante ADP è risultato proporzionale alle dosi dei farmaci impiegati. La seguente scala gerarchica di potenza è disposta in ordine decrescente di attività: pindolol, K 4423, propranolol, INPEA.

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Influence of Vitamin A on Experimental Atherosclerosis in Rabbits

The influence of Vitamin A (alone or in combination with Vitamin E) upon cholesterol induced atherosclerosis has been studied with varying results. WEITZEL et al.[1-3] have reported that both Vitamin A palmitate or Vitamin A palmitate plus Vitamin E exert an anti-atherogenic effect in cholesterol fed rabbits or chickens. This protective effect of the combined vitamins has been confirmed by HEINLEIN and HEINRICH[4] and KÜCHLE and KRUGER[5]. On the other hand, OPPENHEIM and BRUGER[6] reported Vitamin A to be ineffective when administered to cholesterol-fed rabbits, and HORN et al.[7] found that Vitamin A plus E did not inhibit cholesterol induced atherogenesis in the rabbit. BEELER et al.[8] found Vitamin A, but not Vitamin E, to be hypocholesteremic and to inhibit atherosclerosis in chickens fed cholesterol and hydrogenated coconut oil. KINLEY and KRAUSE[9] found Vitamin A to be hypocholesteremic in atherosclerotic, but not in normal patients.

Vitamin A is classified as a lysosomal labilizer[10]. Since the level of lysosomal enzymes is usually elevated in the aortas of rabbits and other species susceptible to experimental atherosclerosis[11,12] we thought it of interest to study the effects of Vitamin A on atherosclerosis and serum-β-glucuronidase levels (a measure of lysosomal activity) in rabbits fed a moderately low level of cholesterol (0.2%) over a period of 1 year.

New Zealand White male rabbits were maintained for 1 year on 100 g/day of either rabbit chow, chow plus 0.2% cholesterol, or chow plus 0.2% cholesterol plus Vitamin A acetate (25 million units per 100 g diet). There were 10 rabbits per group. At sacrifice, the aortas were scored grossly for athreosclerotic involvement by 2 observers working independently. The arch and thoracic portions of the aorta were graded separately on a scale of 0 to 4:0 represents no involvement; 1 represents fatty streaks and small plaques covering less than 10% of the area; 2 indicates 10–25% involvement; 3 indicates 25–50% involvement; 4 indicates more than 50% total involvement.

Serum β-glucuronidase activity was determined by the method of FISHMAN et al.[13] as modified by PLAICE[14]. Incubation was 5 h at 37 °C in 0.2 *M* acetate buffer,

pH 4.5, containing phenolphthalein glucuronide to a final concentration of 0.006 *M*. A unit of β-glucuronidase represents the amount of the enzyme necessary to hydrolyze one μg of the substrate per h under the conditions of assay. Serum cholesterol levels were measured by the method of ZLATKIS et al.[15].

The results are presented in the Table. It is evident that Vitamin A exerted a moderate hypocholesteremic effect (7 to 43%) on rabbits fed 0.2% chloesterol. The severity of the average atherosclerosis was reduced by 31% in the aortic arch and 61% in the abdominal aorta. Half of the rabbits fed Vitamin A plus cholesterol were free of atherosclerotic lesions as were 4 of the 9 rabbits fed cholesterol alone. We have shown that cholesterol feeding increases the level of aortic β-glucuronidase. The level of β-glucuronidase in the sera of rabbits fed cholesterol was no different from that observed in control rabbits: Administration of Vitamin A to cholesterol-fed rabbits significantly reduced serum β-glucuronidase levels suggesting a possible lysosome stabilizing effect of this Vitamin. Since aortic β-glucuronidase levels were not determined, we cannot say if increased levels of this enzyme in the artery would have been reflected in blood levels. The results in the Table (groups B and C), however, suggest that aortic and serum levels of β-glucuronidase may not be correlated.

Further experiments are planned to clarify the role of Vitamin A in the establishment and progression of atherosclerosis[16, 17].

Résumé. La vitamine A a eu un effet hypocholestérimique modéré (7 à 43%) sur des lapins nourris de 0.2% cholestérol. La gravité de l'athérosclérose moyenne fut réduite de 31% dans la crosse aortique, et de 61% dans l'aorte abdominale. L'administration de vitamine A aux lapins nourris de cholésterol a réduit les niveaux de sérum β-glucuronidase d'une manière significative, il est donc possible que cette vitamine ait une influence stabilisatrice sur le lysosome.

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Effect of vitamin A on atherosclerosis and serum β-glucuronidase levels in rabbits

	Group		
	A	B	C
Number	10/10	9/10	10/10
Vitamin A Acetate, 25×10^6 U/100 g diet	+	−	−
Cholesterol, 0.2%	+	+	−
Serum Cholesterol, mg/dl			
Day 125	299 ± 44[a]	323 ± 48	82 ± 5
Day 220	412 ± 45	464 ± 103	111 ± 13
Day 365	219 ± 40	384 ± 74	61 ± 4
Serum β-Glucuronidase (units/ml)			
Day 125	9.6 ± 2.2	13.1 ± 1.9	13.7 ± 1.7
Day 220	7.5 ± 0.9[b]	15.0 ± 1.3	15.3 ± 3.7
Avg. Atheromata			
Arch	1.1 ± 0.5	1.6 ± 0.6	0
Thoracic	0.5 ± 0.2	1.3 ± 0.5	0

[a] Standard error [b] A vs B, $p < 0.001$.

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Influence of Anticholinergic-Antiparkinsonian Agents on the Effects of Narcoanalgesic Drugs in the Rat

There are numerous references in the bibliography about relations between action mechanisms of narcoanalgesic drugs (morphine, meperidine, etc.) and neurohumoral agents (acetylcholine, catecholamines, serotonin, etc.)[1-4]. Experiments carried out in our laboratory also confirmed such relations[5]. In recently performed assays we found also a marked influence of central cholinergic blocking agents (commonly known as antiparkinsonian drugs) on the analgesic and conditioned avoidance suppressing effects of morphine and meperidine in the rat.

Material and methods. The analgesic effect was tested on rats with the method of JANSSEN et al.[6] (Immersion of tail of rats in hot water – in our experiments 51 °C ± 0.5 –, measuring the lapse of time until the tail is withdrawn by the animal). The time between immersion and withdrawal of tail was measured on untreated