lesions by enhancing the activity of hepatic aryl hydrocarbon hydroxylase to a level that is 100 to 800% above that of control animals. With the exception of ethylestrenol, catatoxic steroids are much more effective than the nonsteroidal microsomal enzyme inducer, phenobarbital, in preventing adrenal necrosis elicited by DMBA[4].

Cyproterone acetate is a highly potent catatoxic steroid[1] which is known to cause marked proliferation of the smooth-surfaced endoplasmic reticulum (SER) in rat hepatocytes[5]. Since this synthetic compound is already in clinical use as an antiandrogen[6], it seemed pertinent to study its action on the adrenal lesions produced by DMBA.

Thirty-six female ARS/Sprague-Dawley rats (Madison, Wisconsin, USA), averaging 160 g (range: 150–170 g) and maintained ad libitum on Purina Laboratory Chow and tap water, were divided into 4 groups. Cyproterone acetate [6-chloro-17-hydroxy-1α, 2α-dihydro-2'H-cyclopropa(1, 2)-4, 6-pregnadiene-3, 20-dione acetate (Schering)] was given at the dose of 10 mg/100 g body weight in 1 ml water (homogenized with a trace of Tween 80), twice daily by stomach tube, from the first day of the experiment. DMBA (Eastman) was administered per os at dose levels of 40 mg/100 g body weight, or 40 mg/rat, in 2 ml corn oil, once on the 4th day. Autopsies were performed soon after the animals died, or on the 7th day, when the survivors were killed with chloroform. The adrenals were fixed in a Susa solution (saturated with picric acid), embedded in paraffin, and stained with hematoxylin-phloxine or by the PAS technique. For statistical evaluation, the 'Exact Probability Test' of FISCHER and YATES[7,8] was used.

DMBA produced a 100% incidence of adrenal necrosis at both dose levels (Table). Almost all the animals died when the compound was administered at 40 mg/100 g body weight, whereas the mortality rate was only 25% in the group that received the absolute amount of DMBA (40 mg/rat). Cyproterone acetate significantly reduced the incidence of adrenal lesions and protected against mortality. These results were verified histologically. Extensive adrenocortical necrosis and hemorrhages were seen in the zona fasciculata, and occasionally in the zona glomerulosa, of control rats, confirming the findings of HUGGINS and MORII[9].

The adrenal apoplexy produced by acrylonitrile is not influenced by various catatoxic steroids[10]. In contrast, the adrenocortical lesions elicited by DMBA are prevented by cyproterone acetate. This protection may be due to enhanced hepatic microsomal drug-metabolizing enzyme activity, but biochemical studies will be necessary to verify this hypothesis[11].

Effect of cyproterone acetate on the adrenal lesions produced by DMBA

Group	Treatment[a]	Adrenal necrosis (positive/total)		Mortality (dead/total)	
		I	II	I	II
1	None	10/10	8/8	8/10	2/8
2	Cyproterone acetate	4/10[b]	0/8[c]	1/10[c]	0/8 NS

[a] All animals were given DMBA at the dose of (I) 40 mg/100 g body weight or (II) 40 mg/rat, as described in the text. [b] $P < 0.01$. [c] $P < 0.005$. NS, not significant.

Résumé. L'administration per os d'une dose de 40 mg/100 g de poids corporel ou de 40 mg/rat de DMBA à des rats femelles a produit une nécrose surrénalienne dans 100% des cas. L'acétate de cyprotérone, un stéroïde synthétique très puissant, a réduit le taux des lésions surrénaliennes de façon significative et a empêché la mortalité. L'effet protecteur de ce stéroïde peut être dû à une augmentation de l'activité métabolique des enzymes microsomiales hépatiques.

S. SZABO[12], G. LAZAR and K. KOVACS[13]

Institut de médecine et de chirurgie expérimentales, Université de Montréal, Montréal 101 (Québec, Canada), 30 June 1972.

[4] A. SOMOGYI, K. KOVACS, B. SOLYMOSS, R. KUNTZMAN and A. H. CONNEY, Life Sci. *10*, 1261 (1971).
[5] K. KOVACS, J. A. BLASCHEK, B. D. GARG and A. SOMOGYI, Horm. metab. Res. *3*, 44 (1971).
[6] F. NEUMANN, R. VON BERSWORDT-WALLRABE, W. ELGER, H. STEINBECK, J. HAHN and M. KRAMER, *Recent progress in hormone research 26* (Ed. E. B. ASTWOOD; Academic Press, New York-London 1970), p. 337.
[7] D. J. FINNEY, Biometrika *35*, 145 (1948).
[8] S. SIEGEL, *Nonparametric Statistics for the Behavioral Sciences*, (McGraw-Hill Book Co., Inc., New York-Toronto-London 1965), p. 270.
[9] C. HUGGINS and S. MORII, J. exp. Med. *114*, 741 (1961).
[10] S. SZABO and H. SELYE, Endocr. Exp. *6*, 141 (1972).
[11] This work was supported in part by the Medical Research Council of Canada (Block Term Grant No. MT-1829), the Ministère des Affaires Sociales, Quebec, and was undertaken as a special project of the Council for Tobacco Research, USA, and the Canadian Tobacco Industry. The technical assistance of D. DABOVAL, A. ROKOSZ and B. SALNAVE is gratefully acknowledged.
[12] Fellow of the Medical Research Council of Canada.
[13] Department of Pathology, St. Michael's Hospital, University of Toronto, Toronto, Ont., Canada.

Beta-Blocking Drugs and Human Platelet Aggregation in vitro

Adrenaline, noradrenaline, adenosine diphosphate (ADP) and collagen are considered the agents most effective in causing changes in platelet disc shape and behaviour which induce adhesion and subsequent aggregation. Many authors consider ADP the key-molecule to platelet behaviour both in vivo and in vitro (see review in MUSTARD and PACKHAM[1]).

Over the last 10 years special attention has been paid to connections between thrombotic diseases and platelet aggregation; in fact one of the first steps of thrombus formation is the appearance of platelet aggregates[2,3]. Aggregation-inhibiting drugs are taken into special consideration because they might be potentially useful in conditions of increased thrombogenesis. HAMPTON et al.[2] have observed that several cardiovascular drugs which

[1] J. F. MUSTARD and M. A. PACKHAM, Pharmac. Rev. *22*, 97 (1970).
[2] J. R. HAMPTON, M. J. G. HARRISON, A. J. HONOUR and J. R. A. MITCHELL, Cardiovasc. Res. *1*, 101 (1967).
[3] J. F. MUSTARD and M. A. PACKHAM, Circulation *42*, 1 (1970).

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.

A. GIBELLI, C. MONTANARI, D. BELLANI, V. MANDELLI and G. SACCHETTI[13]

Centro Trasfusionale ed Immuno-ematologico, Ospedale S. Carlo Borromeo, Milano; and Medical Department, Clinical Research Division, and Research Institute, Biometric Division, Carlo Erba, Via C. Imbonati 24, I-20159 Milano (Italy), 20 July 1972.

[4] H. W. BUCHER and P. STUCKI, Experientia *25*, 280 (1969).
[5] K. RYŠÁNEK, C. ŠVEHLA, H. ŠPÁNKOVÁ and M. MLEJNKOVÁ, J. Pharm. Pharmac. *20*, 154 (1968).
[6] G. V. R. BORN and M. J. CROSS, J. Physiol., Lond. *168*, 178 (1963).
[7] G. SACCHETTI, D. BELLANI, C. MONTANARI and A. GIBELLI, Thromb. Diath. Haemorrh. in press.
[8] M. BERGAMASCHI, L. M. FUCCELLA, V. MANDELLI, R. TOMMASINI, C. TURBA and M. M. USARDI, Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. *269*, 447 (1971).
[9] L. M. FUCCELLA, J. REBAN, F. BASSINI, E. LATTES and V. MANDELLI, Europ. J. clin. Pharmac. *4*, 12 (1971).
[10] A. M. KAROW JR., M. W. RILEY and R. P. AHLQUIST, in *Progress in Drug Research* (Ed. E. JUCKER, Birkhäuser Verlag, Basel 1971), vol. 15, p. 103.
[11] K. SAAMELI, Jap. J. Pharmac. Suppl. *22*, 13 (1972).
[12] R. A. TURNER, in *Screening Methods in Pharmacology* (Ed. R. A. TURNER and P. HEBBORN, Academic Press, New York 1971), vol. 22, p. 21.
[13] Reprints by: G. SACCHETTI, Via C. Imbonati 24, I-20159 Milano (Italy).

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,