amounts in urine from rats than in that from rabbits. The presence of this cyclic compound was probably not an artifact formed during treatment as it was not formed from 2-hydroxylaminobenzoic acid added to normal urine.

The main metabolites anthranilic acid, *o*-aminohippuric acid and anthranilic acid ester glucuronide were the same metabolites identified by Charconnet-Harding *et al.* (1953) after the administration of anthranilic acid. The urine of rats and rabbits dosed with 2-hydroxylaminobenzoic acid was free from glucuronic acid or sulphuric acid conjugates of the hydroxylamine since hydrolysis with β -glucuronidase and Taka-diastase failed to liberate free hydroxylamine.

Since *N*-acetylanthranilic acid was excreted in the urine after the administration of anthranilic acid, *N*-hydroxyacetylanthranilic acid would be expected to be present in the urine by analogy with the findings of Cramer *et al.* (1960) in their experiments with 2-acetamidofluorene. However, neither *N*-hydroxyacetylanthranilic acid nor its glucuronide or sulphate could be detected in the urine. Moreover, when *N*-acetylanthranilic acid was incubated with rabbit-liver microsomes (kindly carried out by Dr J. Booth), no 2-*N*-acetylhydroxylaminobenzoic acid or *N*-acetylbenzisoxazolone (if the former cyclizes as it readily does during its preparation) could be detected in the incubation mixture.

SUMMARY

1. 2-Hydroxylaminobenzoic acid appears to be metabolized to products identical with those of anthranilic acid by rabbits. Rats excrete a small proportion of the hydroxylamine unchanged.

2. 2-Hydroxylaminobenzoic acid could not be detected in the urine of rats or rabbits dosed with anthranilic acid or tryptophan.

3. The results give no indication that *N*-hydroxylation of anthranilic acid occurs in rats or rabbits.

A.R.F. thanks the governors of the Royal Marsden Hospital for a Gordon Jacobs Fellowship. The investigation was supported by grants to this Institute from the Medical Research Council, the British Empire Cancer Campaign, the Anna Fuller Fund and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

REFERENCES

- Allen, M. J., Boyland, E., Dukes, C., Horning, E. S. & Watson, G. J. (1957). *Brit. J. Cancer*, **11**, 212.
 Bamberger, E. & Elger, F. (1903). *Ber. dtsh. chem. Ges.* **36**, 1611.
 Bamberger, E. & Elger, F. (1906). *Ber. dtsh. chem. Ges.* **39**, 4252.
 Bamberger, E. & Pyman, F. L. (1909). *Ber. dtsh. chem. Ges.* **42**, 2297.
 Booth, J. & Boyland, E. (1964). *Biochem. J.* (in the Press).
 Boyland, E. & Nery, R. (1962). *Rep. Brit. Emp. Cancer Campgn.* **38**, 50.
 Boyland, E. & Williams, D. C. (1956). *Biochem. J.* **64**, 578.
 Charconnet-Harding, F., Dalglish, C. E. & Neuberger, A. (1953). *Biochem. J.* **53**, 513.
 Cramer, J. W., Miller, J. A. & Miller, E. C. (1960). *J. biol. Chem.* **235**, 885.
 Dalglish, C. E. (1951). *Quart. Rev. chem. Soc., Lond.*, **5**, 227.
 Dalglish, C. E. (1952). *J. chem. Soc.* p. 137.
 Ehrhart, H., Georgii, A. & Stanislawski, K. (1959). *Klin. Wschr.* **37**, 1053.
 Harley-Mason, J. & Archer, A. A. P. G. (1958). *Biochem. J.* **69**, 60f.
 Nelson, N. & Troll, W. (1961). *Fed. Proc.* **20**, 41.
 Uehleke, H. (1963). *Biochem. Pharmacol.* **12**, 219.

Biochem. J. (1964) **91**, 76

The Inhibition of 3 α -Hydroxy Steroid-Activated Nicotinamide-Adenine Dinucleotide (Phosphate) Transhydrogenase by some Antimetabolic Compounds

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(Received 9 September 1963)

The mechanism by which steroid hormones depress tumour growth either experimentally or clinically is not understood, but it is unlikely that they act as simple antagonists. Thus the depression of growth rate of an oestrogen-dependent tumour

with androgens cannot be regarded as a simply physiological antagonism.

One mechanism by which steroid hormones could exert their effect on fast-growing tissues *in vivo* is via the various steroid-activated NAD(P)-transhydrogenating enzyme systems which are known to exist in a number of endocrine-dependent tissues

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and the tumours derived therefrom. Hurlock & Talalay (1958) found that rat liver contained a soluble NAD(P)-transhydrogenating system that can transfer hydrogen from NADPH₂ to NAD in the presence of catalytic quantities of certain 3- α -hydroxy steroids. For the purpose of the present work this system has been assumed to be in a single NAD(P) transhydrogenase and not a complex of enzyme systems (see Hagerman & Vilee, 1959; Jarabak, Adams, Williams-Ashman & Talalay, 1962). In these studies the NAD(P) transhydrogenase from normal rat liver has been used exclusively, but a similar enzyme has been found in human liver, breast and prostate (Baron, Gore & Williams, 1960, 1962).

Timmis (1959, 1961) discussed evidence which led him to the conclusion that a relationship exists between the action of antimetabolic drugs and steroids in the treatment of hormone-sensitive tumours. The nicotinamide nucleotide coenzymes participate in the biosynthetic pathway of nucleic acids via purines and pyrimidines so that the inhibition of NAD(P) transhydrogenase could exert a direct effect on nucleic acid synthesis.

In the present work a preliminary study has been made of the effect of antipurines, antipyrimidines and antagonistic steroid hormones *in vitro* on the 3- α -hydroxy steroid-activated NAD(P) transhydrogenase.

MATERIALS

6-Mercaptopurine monohydrate was obtained from the California Corp. for Biochemical Research, Los Angeles, U.S.A. 6-Azauracil, 6-azauracil riboside, 6-mercaptopurine riboside, 6-amino-2-thiouracil, 5-bromouracil, 2,6-diamino-8-azapurine, 8-aza-adenine, 8-azaguanine, 6-chloropurine, 8-iodopurine, 6-methylmercaptopurine, thioadenine sulphate, 2,6,8-trichloropurine, 8-azahypoxanthine and 8-azaxanthine were from L. Light and Co., Ltd., Colnbrook, Bucks. 2'-Deoxy-5-fluorouridine was from Roche Products Ltd., Welwyn Garden City, Herts. Uridine, inosine, cytidine, guanosine, adenosine, cytidine 5'-monophosphate, guanosine 5'-monophosphate, adenosine 5'-monophosphate, inosine 5'-monophosphate, uridine 5'-monophosphate, androsterone and oestradiol-17 β were from Mann Research Laboratories, New York, U.S.A. Testosterone and stilboestrol were from British Drug Houses Ltd., Poole, Dorset. NAD, NADP (monosodium salt), glucose 6-phosphate (monosodium salt) and glucose 6-phosphate dehydrogenase (specific activity approx. 70 m-moles of substrate oxidized/min./mg. of enzyme protein) were from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. The drugs were dissolved in 0.1M-tris-HCl buffer, pH 7.5. Compounds insoluble in 0.1M-tris-HCl buffer, pH 7.5, were dissolved by shaking in either 0.1N-sulphuric acid or 0.1N-ammonia and the pH was adjusted to 7.5. 6-Mercaptopurine in solutions at pH values between 7 and 8 was estimated spectrophotometrically by using extinction coefficients to determine the final concentration of the drug.

METHODS

Preparation from rat-liver of 3- α -hydroxy steroid-activated nicotinamide-adenine nucleotide (phosphate) transhydrogenase. Male rats were anaesthetized with ether and their livers perfused *in vivo* with 0.9% sodium chloride solution containing heparin (approx. 5 units/ml.). The perfusion was carried out by inserting the needle of an intravenous-infusion apparatus into the hepatic artery. After 2 min. the hepatic vein was cut and the perfusion continued until most of the blood had been removed from the liver. The liver was then removed, cooled in ice, cut into small pieces and suspended in an ice-cold solution of nicotinamide (10 mM), cysteine (1 mM) and EDTA (1 mM) in glass-distilled water containing 20% (v/v) of glycerol (AnalaR grade), 1 ml. of homogenizing medium being used per g. of rat liver. The suspension was treated by an Ultra-Turrax homogenizer for 10 sec. with cooling. The homogenate was ultracentrifuged (at 105 000g for 1 hr.) and the clear supernatant solution removed and maintained at 0°.

The extract was precipitated by the dropwise addition of ice-cold saturated ammonium sulphate and was collected by centrifugation (at 37 000g for 30 min.). The ammonium sulphate (AnalaR grade) had previously been purified by recrystallization from hot water, the saturated ammonium sulphate solution being prepared and neutralized with aq. ammonia. The precipitated fraction was dissolved in a minimum of distilled water, transferred to a dialysis bag made from Visking tubing and dialysed for 15-18 hr. against two changes of distilled water (2 l.) containing tris-HCl buffer, pH 7.4 (0.5 mM) and EDTA (100 mg.) at 0°. The suspension obtained from the dialysis bag was centrifuged (at 50 000g for 10 min.) and the clear supernatant divided into 5 ml. portions which were stored at -20°. Each 5 ml. portion was heated rapidly to 0° immediately before use. It was observed that, although the enzyme can be stored for some months at -20° with very little loss of activity, repeated freezing and thawing must be avoided.

Enzyme assays. The assays were carried out on a Hilger Uvispek spectrophotometer, with automatic recording, at room temperature. In the preliminary screening experiments four tubes were used, each containing the following reagents: 0.10 ml. of 0.1M-tris-HCl buffer, pH 7.5; 0.01 ml. of glucose 6-phosphate dehydrogenase diluted to 1 mg. of protein/ml. with 3.3M-ammonium sulphate solution, pH 7 (Baron, Gore, Pietruszko & Williams, 1963); 0.05 ml. of 0.06M-glucose 6-phosphate (disodium salt); 0.10 ml. of 1 mM-NADP (monosodium salt).

To each of tubes 1 and 2 was added 0.01 ml. of acetone-water (1:1, v/v), and to each of tubes 3 and 4 0.01 ml. of a solution of androsterone (1 μ g./0.01 ml.) in acetone-water (1:1, v/v). A further 0.1 ml. of 0.1M-tris-HCl buffer, pH 7.5, was added to tube 1, and 0.1 ml. of a solution of the enzyme was added to each tube; at zero time 0.1 ml. of 7.5 mM-NAD was added to each of tubes 2, 3 and 4, tube 1 serving as a blank. The solutions were transferred as quickly as possible to microcuvettes (capacity 0.3 ml.) with 1 cm. light-paths, and the extinctions at 340 m μ were measured continuously at 30 sec. intervals for 15-30 min. in a Hilger Uvispek spectrophotometer with a Gilford automatic recording attachment (Gilford Instrument Laboratories Inc., Oberlin, Ohio, U.S.A.), which allows each of the four solutions to be measured consecutively. All reaction rates were calculated from the slopes of the initial

(linear) portions of the graphs. In the preliminary screening experiments the effect of the drug on the reduction of NAD in the absence of added steroid was neglected. In the later experiments drug was added to tubes 3 and 4 and the solution of the steroid to tubes 2 and 4, a preliminary experiment having been carried out to determine the reduction of NAD in the absence of added steroid. In experiments with fixed concentrations of 6-mercaptopurine and different concentrations of a component of the reaction mixture a preliminary experiment to determine the reduction of NAD in the absence of added steroid was carried out each time that experimental conditions were changed. Considerable difficulty was experienced in obtaining consistent results when drugs absorbed strongly at 340 m μ . It was, however, possible, in certain cases, to put the drug in the blank and to 'back off' the resulting background extinction. This technique is limited by the relative insensitivity of the instrument at high slit-widths.

Determination of protein concentrations. These were determined by measurement of the extinctions at 280 and 260 m μ , the protein concentration (in mg./ml.) being calculated by the formula $1.5E_{280} - 0.75E_{260}$.

RESULTS AND DISCUSSION

In the preliminary screening experiments a group of compounds was tested for ability to inhibit 3 α -hydroxy steroid-activated NAD(P) transhydrogenase *in vitro*. Two groups of anti-metabolites were investigated: the inhibitory effect of purine analogues is shown in Table 1 and that of pyrimidine analogues in Table 2. The results quoted are average inhibitions obtained from several determinations. In the first group 6-mercaptopurine was easily the most active compound tested under the standard experimental conditions and the riboside of 6-mercaptopurine is also an effective inhibitor. Of the group of pyrimidine analogues tested 6-azauracil and its riboside were the most active compounds, although the inhibition produced was considerably less than that of 6-mercaptopurine. Although a number of other analogues of both purines and pyrimidines do inhibit the NAD(P)-transhydrogenase system, in no case has inhibition been produced by a normal purine or pyrimidine metabolite. A systematic examination of the relationship between the NAD(P)-transhydrogenase system and either 6-mercaptopurine or 6-azauracil was then undertaken. The inhibition obtained with each of these substances under the same experimental conditions as used for the preliminary screening but at various pH values, in 0.1M-tris buffer, is shown in Fig. 1. The pH-inhibition curves of the ribosides are similar in shape to those of the parent compounds. The optimum inhibition as a percentage of the control NAD(P)-transhydrogenase activity was calculated over the pH range 6.0–8.6. The optimum pH for inhibition with 6-mercaptopurine appears to be significantly higher than that for 6-azauracil, but

both optima are sufficiently near pH 7.5, which is the optimum pH for the 3 α -hydroxy steroid-activated NAD(P) transhydrogenase, to allow this value to be used throughout the present work.

Table 1. *Inhibition of 3 α -hydroxy steroid-activated nicotinamide-adenine dinucleotide (phosphate) transhydrogenase by purine derivatives*

Experimental details are given in the text. Results are corrected to the nearest 5% and the method is reproducible within these limits. The following purine analogues gave no inhibition when tested in the same system: 8-azaguanine, 6-iodopurine and 2,6-diamino-8-azapurine, each at a concentration of 100 μ g./test; inosine, inosine 5'-monophosphate, guanosine and guanosine 5'-monophosphate, each at a concentration of 50 μ g./test.

Compound	Concn. of compound (μ g./test)	Average inhibition (%)
6-Mercaptopurine	30	55
	10	15
6-Mercaptopurine riboside	50	35
	10	10
2,6,8-Trichloropurine	50	30
	10	10
8-Aza-adenine	100	10
	50	5
Thioadenine	50	10
	10	0
8-Azaxanthine	100	10
	10	0
6-Chloropurine	100	5
	50	0
8-Azahypoxanthine	100	5
	50	0
6-Methylmercaptopurine	100	5
	10	0
Adenosine	50	5
Adenosine 5'-monophosphate	50	5

Table 2. *Inhibition of 3 α -hydroxy steroid-activated nicotinamide-adenine dinucleotide (phosphate) transhydrogenase by pyrimidine derivatives*

Experimental details are given in the text. Results are corrected to the nearest 5% and the method is reproducible to within these limits. The following pyrimidine analogues gave no inhibition when tested in the same system: 2-deoxy-5-fluorouridine and 4-amino-2-thiouracil, each at a concentration of 100 μ g./test; uridine, uridine 5'-monophosphate, cytidine, and cytidine 5'-monophosphate, each at a concentration of 50 μ g./test.

Compound	Concn. of compound (μ g./test)	Average inhibition (%)
6-Azauracil	100	30
	30	15
6-Azauracil riboside	100	20
	30	10
5-Bromouracil	100	10
	50	5

The effect on the percentage inhibition of varying the individual constituents of the test system separately has been determined for both mercaptopurine and azauracil. As the changes in inhibition with these variations are essentially similar with both purine and pyrimidine analogues, only those obtained with 6-mercaptopurine have been recorded (Table 3). Expt. 1 of Table 3 shows the effect of varying the amount of transhydrogenase measured as mg. of total protein. The percentage inhibition

appears to be approximately proportional to the quantity of enzyme present, which suggests that the inhibition is not due to direct interaction between the mercaptopurine and the enzyme. Solutions containing 3–5 mg. of protein in 0.1 ml. were therefore used as a source of enzyme in NAD(P)-transhydrogenase estimations in subsequent work.

Expt. 2 of Table 3 shows the relationship between the quantity of steroid present and the inhibition produced. Over the enzyme range used there is no variation in inhibition. This could be explained by assuming that there is no interaction between the steroid and 6-mercaptopurine, although this cannot be regarded as proof. In subsequent experiments a concentration of 11 μ M has been used.

Expt. 3 of Table 3 shows the effect of varying the NADP concentration in the test medium. The percentage inhibition of NAD(P) transhydrogenation appears to increase as the concentration of the NADP decreases. This suggests that there is some interaction between the NADP and the 6-mercaptopurine under these conditions. There is little change in percentage inhibition with increase in NADP concentration above 270 μ M, so that this concentration was used in subsequent experiments.

Expt. 4 of Table 3 shows the effect of changing the quantity of glucose 6-phosphate dehydrogenase in the test solution. It has been shown by Vogel, Snyder & Schulman (1963) that this enzyme can be inhibited by antimetabolites of folic acid and the enzyme is also inhibited by 6-mercaptopurine and azauracil. There is, however, no change in percentage inhibition when the enzyme is increased to a concentration of 3 mg. of protein or more per test.

Fig. 1. Effect of the pH (in 0.1M-tris buffer) of the test solution on the inhibition of NAD(P) transhydrogenation by antimetabolites. O, Test solution contained 6-mercaptopurine (250 μ M); Δ , test solution contained 6-azauracil (310 μ M). Inhibition is expressed as a percentage of the NAD(P) transhydrogenation produced in a test solution at the same pH containing no inhibitor.

Table 3. Effect of changing the final concentrations of constituents of the reaction mixture on the nicotinamide-adenine dinucleotide (phosphate) transhydrogenase by 6-mercaptopurine

Experimental details are given in the text.

Expt. no.	Concn. of 6-mercaptopurine (μ M)	Concn. of transhydrogenase (mg. of total protein/test)	Concn. of androsterone (μ M)	Concn. of NADP (μ M)	Concn. of glucose 6-phosphate dehydrogenase (mg. of total protein/test)	Inhibition (%)
1	280	7.2	11	270	3.0	30
	280	5.0	11	270	3.0	25
	280	3.6	11	270	3.0	15
	280	2.5	11	270	3.0	8
	280	1.2	11	270	3.0	6
2	300	4.7	33	300	3.0	35
	300	4.7	11	300	3.0	34
	300	4.7	5	300	3.0	32
3	280	3.5	11	680	3.0	15
	280	3.5	11	270	3.0	16
	280	3.5	11	140	3.0	23
	280	3.5	11	30	3.0	33
4	240	4.4	11	270	10.6	29
	240	4.4	11	270	5.0	30
	240	4.4	11	270	3.0	31
	240	4.4	11	270	1.5	23
	240	4.4	11	270	0.5	10

This quantity of glucose 6-phosphate dehydrogenase was therefore used in the standard test solution.

With the test system established above, the inhibitory effect of 6-mercaptopurine and of its riboside were investigated at various concentrations of the inhibitor, and the results obtained from two such sets of experiments are shown in Fig. 2. In these experiments the effect of 6-mercaptopurine itself is about twice that of the corresponding riboside when expressed as a molar concentration in the final volume of test solution. The inhibitory effect of 6-azauracil and its riboside are shown in Fig. 3. The inhibitions produced by these com-

Fig. 2. Effect of changing the final concentration of purine analogue on the percentage inhibition of NAD(P) transhydrogenation. The antimetabolite was: ○, 6-mercaptopurine; ●, 6-mercaptopurine riboside.

Fig. 3. Effect of changing the final concentration of pyrimidine analogue on the percentage inhibition of NAD(P) transhydrogenation. The antimetabolite was: △, 6-azauracil; ▲, 6-azauracil riboside.

pounds, even at the higher concentrations used, are considerably less than those of 6-mercaptopurine. The difference in inhibition between 6-azauracil and its riboside is also proportionately much less than that between 6-mercaptopurine and its corresponding riboside.

The question of possible synergism between purine and pyrimidine analogues in the suppression of NAD(P) transhydrogenation has been investigated. If it is assumed that NAD(P) transhydrogenation plays a part in controlling the growth rate of steroid-dependent tumours, then any synergistic effect would be most important in view of the toxicity of both types of antimetabolic compound. Fig. 4 illustrates one of a number of these experiments which were performed at a range of different drug concentrations by varying the relative concentrations of the two antimetabolites. Curve *D* was obtained by adding molar equivalent amounts of both 6-mercaptopurine and 6-azauracil to the NAD(P)-transhydrogenase system. Although these compounds produce an additive effect under all conditions so far tried, there has been no evidence of synergism between the two types of antimetabolite. Similar results have been obtained with other members of the groups.

A further question of importance from a physiological viewpoint is the interaction between antimetabolic substances and the 'antihormones'. It is known, for example, that in breast tumours, which are dependent to some extent on androgens, a slowing of growth rate can be produced by the

Fig. 4. Inhibition of NAD(P) transhydrogenation produced by 6-mercaptopurine and 6-azauracil separately and in combination. *A*, Transhydrogenation with androsterone (11 μM); *B*, transhydrogenation with androsterone (11 μM) and 6-azauracil (250 μM); *C*, transhydrogenation with androsterone (11 μM) and 6-mercaptopurine (250 μM); *D*, transhydrogenation with androsterone (11 μM), 6-azauracil (250 μM) and 6-mercaptopurine (250 μM); *E*, basal transhydrogenation in the absence of steroid. The graphs represent experimental curves in which readings were taken automatically over 5 sec. intervals every 20 sec.

administration of oestrogens. These tumours can sometimes be further treated with antimetabolites, so that it was decided to determine the effect of such a system *in vitro* under these conditions. The 3 α -hydroxy steroid-activated NAD(P) transhydrogenase can be inhibited by oestradiol, stilboestrol, testosterone and several other types of hormone (Koide, Chen & Freeman, 1960; Williams, Baron & Gore, 1960). The interaction between these substances and 6-mercaptopurine or 6-azauracil has been examined with the present test system. The response of each pair of inhibitors (i.e. hormone and antipurine or antipyrimidine) is essentially the same; the case of oestradiol-17 β and 6-mercaptopurine is presented (Fig. 5) as being typical of this group of experiments. These experiments show that additive inhibitions can be produced by combinations of steroid hormones and purine or pyrimidine analogues, although in no case was true synergism obtained. A rather similar picture can be seen when stilboestrol or testosterone is substituted for oestradiol-17 β . A similar effect can also be observed by using the ribosides instead of 6-mercaptopurine and 6-azauracil. There is much clinical evidence and evidence from experimental tumours which

leads to the conclusion that many tumours, especially those of endocrine origin, can be controlled by steroid treatment. The involvement of the steroid-dependent NAD(P) transhydrogenases in such treatment was suggested by Hollander, Jonas & Smith (1958) as a result of their work on human breast cancer. If this is the case then combined treatment with antagonistic hormones and antimetabolites should give an additive effect. This treatment, in suitable cases, should decrease the toxicity of the drug to normal tissue since a correspondingly lower dose of antimetabolite should be required to produce an effect. Much experimentation *in vivo* will be required to establish the relevance of steroid-dependent NAD(P) transhydrogenation in the antimetabolic treatment of malignant tissues.

SUMMARY

1. This work establishes the inhibition *in vitro* of the 3 α -hydroxy steroid-activated NAD(P)-transhydrogenating enzyme system from rat liver by certain derivatives of purines and pyrimidines.

2. Of the compounds tested the most active purine and pyrimidine analogues appear to be those having a high therapeutic index against experimental cancer.

3. The inhibition produced is additive as between purines and pyrimidines and also between inhibitory steroid hormones and the antimetabolites.

REFERENCES

- Baron, D. M., Gore, M. B. R., Pietruszko, R. & Williams, D. C. (1963). *Biochem. J.* **88**, 19.
 Baron, D. M., Gore, M. B. R. & Williams, D. C. (1960). *Acta endocr., Kbh.*, **35**, 981.
 Baron, D. M., Gore, M. B. R. & Williams, D. C. (1962). *Proc. 8th int. Cancer. Congr., Moscow*, p. 107.
 Hagerman, D. D. & Vilee, C. A. (1959). *J. biol. Chem.* **234**, 2031.
 Hollander, V. P., Jonas, H. & Smith, D. E. (1958). *Cancer*, **11**, 803.
 Hurlock, B. & Talalay, P. (1958). *J. biol. Chem.* **233**, 886.
 Jarabak, J., Adams, J. A., Williams-Ashman, H. G. & Talalay, P. (1962). *J. biol. Chem.* **237**, 345.
 Koide, S. S., Chen, C. & Freeman, S. (1960). *Biochim. biophys. Acta*, **38**, 374.
 Timmis, G. M. (1959). In *Cancer*, vol. 6, p. 1. Ed. by Raven, R. W. London: Butterworth and Co. Ltd.
 Timmis, G. M. (1961). *Advanc. Cancer Res.* **6**, 369.
 Vogel, W. H., Snyder, R. & Schulman, M. P. (1963). *Biochem. biophys. Res. Commun.* **10**, 1.
 Williams, D. C., Baron, D. M. & Gore, M. B. R. (1960). *Biochem. J.* **76**, 37p.

Fig. 5. Inhibition of androsterone activation of NAD(P) transhydrogenation by oestradiol-17 β alone and in combination with 6-mercaptopurine and 6-azauracil. A, transhydrogenation with androsterone (11 μ M) and oestradiol-17 β (50 μ M); C, transhydrogenation with androsterone (11 μ M), oestradiol-17 β (5 μ M) and 6-azauracil (250 μ M); D, transhydrogenation with androsterone (11 μ M), oestradiol-17 β (5 μ M) and 6-mercaptopurine (250 μ M); E, basal transhydrogenation in the absence of steroid. The graphs represent experimental curves in which readings were taken automatically over 5 sec. intervals every 20 sec.