J. Pharm. Pharmac. 1972, **24**, 593–601 *Received March* 2, 1972

Synergistic action of pyrazole on ethanol incoordination: differential metabolic and central nervous system effects

L. GOLDBERG, C. HOLLSTEDT, A. NERI AND U. RYDBERG

Department of Alcohol Research, Karolinska Institutet, S-104 01 Stockholm, Sweden

The influence of pyrazole on ethanol-induced incoordination was measured by a modified tilting-plane technique. Pyrazole (1·77 mmol/kg; 120 mg/kg) and/or ethanol (32·6 mmol/kg; 1·5 g/kg) was given intraperitoneally to rats. Impairment of coordination was related to blood ethanol concentrations. The mean maximal impairment was significant in all conditions. For ethanol alone the maximal impairment was 8·7%, for pyrazole alone 4·8% and for ethanol + pyrazole 16·5%. Ethanol alone induced a total impairment, assessed planimetrically, of 530 units. Pyrazole alone induced an impairment gradually increasing with time (totally 1070 units). When pyrazole was combined with ethanol, the rate of ethanol elimination was reduced by 81%, and the time it remained in the blood was prolonged from 196 ± 11 to 850 ± 16 min. The rate of disappearance was reduced from 8·05 to 1·57 μg/ml per min. The total impairment increased to 5600 units, indicating a synergistic interaction between ethanol and pyrazole, which is contrary to the normalizing effects of pyrazole on ethanol-induced metabolic changes. A "post-drug" impairment was observed one week after pyrazole, while no such effects were found after repeated administration of saline or ethanol alone in control animals. Thus, pyrazole showed acute and long-term toxic effects.

Ethanol has two main effects, one metabolic involving energy transfer, the other pharmacodynamic interfering with some physiological processes, both central and peripheral.

Interaction with ethanol metabolism may be elicited in a number of ways. Theorell & Yonetani (1963) found pyrazole to be a potent inhibitor *in vitro* of liver alcohol dehydrogenase (LADH). An inhibition of ethanol metabolism *in vivo* has been found in some species (Lester, Felzenberg & Keokosky, 1968; Goldberg & Rydberg, 1969; Reynier, 1969; Watkins, Goodman & Tephly, 1969; Goldberg & Ström, 1970: Goldstein & Pal, 1971).The effect of pyrazole on zinc-containing enzyme systems led to the inference that the inhibition of LADH was specific (Theorell, 1965; Reynier, 1969). But it has toxic effects as well as actions on other enzyme systems, e.g. microsomal hydroxylases (Kiessling & Rydberg, 1969; Lelbach, 1969; Benson, 1970; Rubin, Gang & Lieber, 1970; Lieber, Rubin & others, 1970).

Pyrazole and its derivatives have been used experimentally to block various metabolic effects of ethanol. The decrease of the NAD^+ : NADH ratio in rat liver, elicited by ethanol, was partially counteracted when pyrazole was combined with ethanol (Bustos, Kalant & others, 1970). The ethanol-induced increase in the lactate: pyruvate ratio in human blood was reduced by 4-methylpyrazole (Blomstrand & Theorell, 1970). The inhibiting influence on gluconeogenesis by ethanol was less marked after pyrazole (Krebs, Freedland & others, 1969). The increase in liver

triglycerides induced by ethanol administration was partially counteracted by pyrazole (Bustos & others, 1970; Johnson, Hernell & others, 1971), and by 4-methylpyrazole (Blomstrand & Forssell, 1971). However, Morgan & Di Luzio (1970) could not confirm that a decrease in liver triglycerides occurred after pyrazole. The ethanol-induced increase in the ratio of 17-hydroxysteroids:17-ketosteroids in liver slices reduced by 4-bromopyrazole, was reflected in a shift in the redox pair of 5-androstene-3β-17β-diol:3β-hydroxy-5-androstene-17-one (Admirand, Cronholm & Sjövall, 1970).

The effects of ethanol on the central nervous system have been attributed to a primary effect of ethanol itself. A close correlation has been found of changes in the central nervous functions and in blood ethanol concentration (Goldberg, 1943; Goldberg, 1966; Wallgren, 1966; Kalant, 1970). It has been suggested that ethanol-induced effects in the central nervous system are secondary and are brought about by metabolites such as acetaldehyde (Kiessling, 1962), or are due to ethanol-induced metabolite changes, e.g. redox shifts, among others mediated through the NAD^+: NADH and $NADP^+$: NADPH systems and reflected for example in the change in the lactate : pyruvate ratio (Kalant & Khanna, 1969).

Rats after the administration of pyrazole + ethanol may display greater and more prolonged behavioural disturbances than animals receiving ethanol alone (Goldberg & Rydberg, 1969; Morgan & Di Luzio, 1970). Recently, Blum, Geller & Wallace (1971) have demonstrated the synergistic effect of pyrazole and 3-methylpyrazole with ethanol in performance tests in rats and mice. Pyrazole prolongs the sleeping-time induced by hexobarbitone (Reynier, 1970). Feldstein & Kurcharski (1971) have shown that both pyrazole and ethanol enhance tryptophol-induced sleep in mice.

We wished to find if pyrazole had any central depressant effects, or modified ethanol-induced effects on the central nervous system, and to correlate the possible degree of change to the concentration of ethanol in blood, and we also set out to ascertain the mechanisms of action of ethanol on coordination and behaviour, whether primary and induced by ethanol itself, or secondary and induced by metabolites or by metabolic effects.

METHODS

Material. Fifteen male rats of the Sprague-Dawley strain, 160 $\pm$ 6 g (mean $\pm$ s.e.) divided into three sub-groups, were tested in the main series under three different drug conditions and one post-drug condition. Sixteen animals were used as controls. A further 16 rats were used to elucidate various aspects of the technique.

Chemicals. Ethanol (Vin & Spritcentralen Ltd.) 95% w/v, diluted with 0·9% saline to a concentration of 11·3% w/v. The ethanol was injected intraperitoneally in a standard dose of 1·5 g/kg (32·6 mmol/kg). Pyrazole (Fluka Ltd.) in a 2% w/v solution in saline was injected intraperitoneally 15 min before the ethanol in a standard dose of 120 mg/kg (1·77 mmol/kg).

Blood sampling. 10 μl blood samples were withdrawn in duplicate from the tip of the tail at 30, 60, 90 and 120 min after the administration of ethanol, and thereafter at 60 min intervals for a total of 10 h. The samples were transferred to 2 ml Auto Analyzer cups with 0·9 ml sodium fluoride solution, 0·2% w/v.

Ethanol determination. The concentration of ethanol was measured enzymatically by an autamated dialysis yeast ADH method (Goldberg & Rydberg, 1966) using 10 μl

samples at a rate of 50–60 samples/h. The s.e. of duplicate samples was ±0·019 mg ethanol/ml blood.

Ethanol parameters and degree of inhibition. For definitions of ethanol parameters, see Widmark (1930). Degree of inhibition =

$$= \frac{\beta \text{ control} - \beta \text{ pyrazole}}{\text{control}} \quad (1); \text{ or } \frac{\beta r \text{ control} - \beta r \text{ pyrazole}}{\beta r \text{ control}} \quad (2),$$

where β is the rate of disappearance in μg/ml per min, and βr is the total amount of ethanol metabolized per time unit in mg/kg per min.

Determination of changes in coordination. Principles of the technique using a tilting plane, were given by Arvola, Sammalisto & Wallgren (1958). In our modification, the surface of hard board rough side up, was surrounded by a transparent plastic wall, 11 cm high and curved in front, with a piece of foam rubber attached at the bottom of the plane. The rat was placed head towards the curved front and the plane pulled by a string connected to an electric motor, and tilted at a constant speed from 0 to 90° in 8 s. When the animal started to slide, the tilting was stopped and the angle was recorded. The procedure was repeated four times at each trial. A statistical analysis showed that the s.e. based on scores from 15 animals ranged from 0·63 degrees under the pre-drug control condition to 0·96 degrees under drug conditions, corresponding to 1·0 to 1·7 % of the mean.

Design. The main test series comprised four conditions for each group of 5 animals tested (n = 15). (1) Pyrazole + saline, (2) ethanol + saline, (3) ethanol + pyrazole and (4) saline + saline (post-drug control). Conditions 1–3 were rotated in a random order. One week passed between each condition.

In the control series (n = 16) the animals were tested twice in two 10 h sessions, separated by one week, to study the behaviour of intact, naive animals and also the effect of repetitive measurements.

The animals had free access to tap water and pelleted food until 1 h before experiments which were made at the same time of day.

An experimental session started with two initial pre-drug trials of tilting, 30 min apart, to obtain baseline values. Ten min after the second pre-drug trial pyrazole (or saline) was administered, and 15 min later the ethanol (or saline). Trials were then carried out at 30, 60, 90 and 120 min after the ethanol, and then at hourly intervals for a total of 10 h. Each trial was followed by blood sampling. Blood was also taken in the control and pyrazole conditions when no ethanol was given.

RESULTS

Blood ethanol concentration

Control (saline). No measurable ethanol concentration was found in peripheral blood.

Ethanol. The blood ethanol curve reached a maximum of 1·33 mg/ml blood 30 min after administration. The decline of the curve was rectilinear (Fig. 1). The rate of decline averaged 8·05 μg ethanol/ml blood per min. The ethanol concentration was zero at 196 min. Means and s.e. for blood ethanol parameters are presented in Table 1, and mean blood ethanol curves are shown in Fig. 1B.

Pyrazole. No measurable ethanol concentration was found in peripheral blood.

Ethanol + pyrazole. The blood ethanol curve after administration of ethanol + pyrazole showed a higher maximum value and a much lower rate of disappearance

than after ethanol alone. The decline was curvilinear (Fig. 1B). The rate of decline averaged 1·57 μg ethanol/ml blood per min. The ethanol was eliminated after 851 min.

The degree of inhibition (mean $\pm$ s.e.) brought about by the pyrazole dose used, 120 mg/kg at an ethanol dose of 1·5 g/kg, was 80·6 $\pm$ 1·4% calculated from rates of decline (β, formula 1), and 76·5 $\pm$ 0·8% for the turnover rates (βr, formula 2).

Change in performance (coordination) under various conditions

Control (saline). The performance of intact animals showed only slight variations during the first 4 h. The initial sliding angle was 62·6 $\pm$ 0·62 degrees. A slight

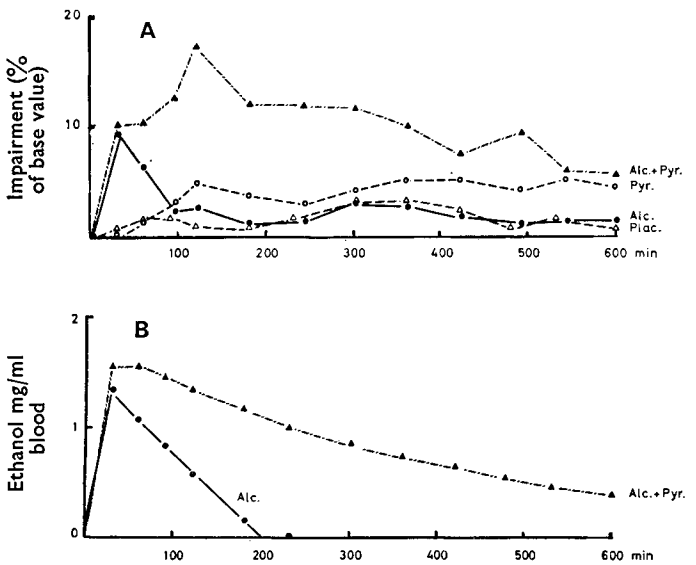

FIG. 1. A. Change with time in degree of impairment (changes in sliding angles in per cent of initial pre-drug values determined by an automated tilting-plane technique) after pyrazole (120 mg/kg) and/or ethanol (1·5 g/kg).
B. Blood ethanol curves after ethanol (1·5 g/kg), lower curve, and ethanol (1·5 g/kg) + pyrazole (120 mg/kg), upper curve. n = 15.

impairment in coordination was then noted (maximally 3·4%), lasting for some 3–4 h. The performance returned to the initial level at 7–8 h after the beginning of the experiment (Fig. 2).

The drug conditions. The effects of the drugs were evaluated by comparing the performance scores during each of the experimental conditions with the scores in the control group. All the post-drug measurements in each single animal were expressed as deviations from the mean of the baseline values obtained in the pre-drug trials. To make a comparison possible between different series, the deviations found in each of the drug conditions were expressed in per cent of the pre-drug values. The initial values showed only random variations. The values obtained are shown in Fig. 1A

Ethanol. Ethanol induced an impairment reaching a maximum simultaneously with the blood ethanol maximum. The performance then improved parallel with the decline in blood ethanol (Fig. 1B). The impairment could still be detected at 133 min. The blood concentration then was 0·52 mg ethanol per ml (Fig. 1B). This

Table 1. *Effect of pyrazole on blood ethanol parameters in the rat.* (n = 15). 1·5 g/kg (32·6 mmol/kg) ethanol intraperitoneally, with or without the addition of 120 mg/kg (1·77 mmol/kg) pyrazole. Mean ± s.e.

Blood ethanol parameters	Ethanol	Ethanol + pyrazole	Significance of difference
Maximal blood ethanol concn (mg/ml)	1·33 + 0·072	1·61 ± 0·046	$P < 0·01$
Time till blood ethanol became zero (min)	196 ± 8·0	851 ± 16·2	$P < 0·001$
Rate of disappearance ($\beta \times 10^3$ μg/ml per min)	8·05 ± 0·27	1·57 ± 0·051	$P < 0·001$
Total amount of ethanol metabolised per time unit (mg/kg per min βr) ..	7·84 ± 0·32	1·77 ± 0·035	$P < 0·001$

value represents a "threshold" value i.e. the blood ethanol concentration at which performance returns to normal. The small fluctuations seen after the main impairment had disappeared were also seen in the control situation (Fig. 2), as well as after ethanol + pyrazole.

Pyrazole. The performance after administration of pyrazole was characterized by an increasing impairment, evident already at 120 min and then progressing gradually, reaching a maximum of 4·8% at 10 h, at the time when the experiment was terminated (Fig. 1). The progressive increase in impairment with time was proved to be statistically significant by regression and correlation analysis. The correlation coefficient was r = 0·97 ± 0·042 ($P < 0·001$), and the regression coefficient was b = 0·48 ± 0·021 ($P < 0·001$). The mean values of the consecutive measurements in the pyrazole condition were compared by *t*-tests to the corresponding values obtained in the control group. The differences were highly significant ($P < 0·001$).

Ethanol + pyrazole. The addition of pyrazole to the ethanol brought about an impairment which greatly exceeded that induced by ethanol alone. The initial increase in impairment was similar to that after ethanol alone. Then it further increased and reached a maximum twice that produced by ethanol alone, 16·5% against 8·7%. This peak effect occurred later than the peak of ethanol concentration.

The decline of the impairment was gradual. When the experiment was terminated after 10 h the performance had not returned to the pre-drug value but was still 6·4%. At this time there was still ethanol left in the blood (0·31 mg/ml) (Fig. 1).

Analysis of the differences between conditions

Sliding angle. The differences between the drug conditions were submitted to two-tailed *t*-tests, using intra-pair differences. For this purpose the experimental values were expressed as deviations from baseline values for each condition. The

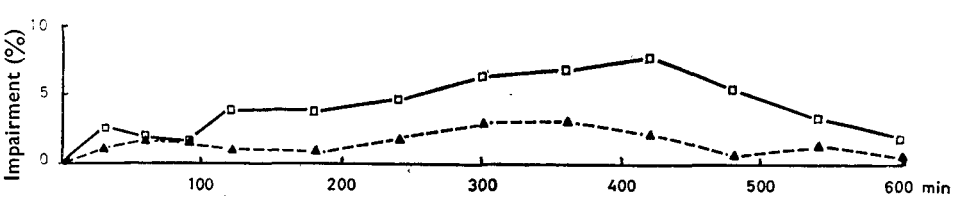

FIG. 2. Change in degree of impairment with time in control animals and in the post-drug controls subjected to tilting 1 week after the main series (n = 16). Difference between the two series of animals significant (P < 0·01). □ Post-drug controls ▲ Controls (no drug).

results showed that significant differences were obtained between ethanol and pyrazole conditions in the first drug trial ($P < 0{\cdot}001$), between ethanol + pyrazole and pyrazole for each of the first seven trials ($P < 0{\cdot}001$), and between ethanol + pyrazole and ethanol at each of the six measurements between 90 and 360 min after the ethanol administration, ranging from $P < 0{\cdot}01$ to $P < 0{\cdot}001$.

Area. As one expression of the over-all impairing effect, the area of impairment (% impairment × min) was calculated planimetrically. The impairment in the control condition corresponded to an area of 150 units. In the ethanol condition the area was 530 units and in the pyrazole condition 1070 units. The corresponding area for the ethanol + pyrazole condition was 5600 units, hence a more than 10-fold increase, when corrected for the impairment in the control condition. The corresponding areas of the "active" part of the ethanol curve (Goldberg 1943, 1966)—that over the threshold of 0·52 mg ethanol/ml—were in the ethanol condition 54 units (concentration in mg per ml × min) and 575 units in the ethanol + pyrazole condition, thus a 10-fold increase.

Correlation of degree of impairment and concentration of ethanol in blood

To assess whether the decrease in impairment was related to the decrease in the blood ethanol curve, the mean performance scores, i.e. the degree of impairment corrected for the values found in the control condition, were plotted against the corresponding mean blood ethanol values, for the ethanol as well as the ethanol + pyrazole conditions, starting with the peak values.

Rectilinear regressions were found. The slope in the ethanol condition was $b = 9{\cdot}66 \pm 3{\cdot}01$ ($P<0{\cdot}01$), and in the ethanol + pyrazole condition $b = 11{\cdot}1 \pm 1{\cdot}63$ ($P < 0{\cdot}001$). The correlation coefficients were $r = 0{\cdot}915 \pm 0{\cdot}285$ ($P < 0{\cdot}01$) and $r = 0{\cdot}932 \pm 0{\cdot}137$ ($P < 0{\cdot}001$) respectively.

The x-intercepts, i.e. the threshold value at which performance returns to the pre-drug value, were $0{\cdot}52 \pm 0{\cdot}17$ mg ethanol/ml blood in the ethanol condition, being different from zero ($P < 0{\cdot}001$) and $-0{\cdot}05 \pm 0{\cdot}13$ mg per ml in the ethanol + pyrazole condition, not differing from zero ($P < 0{\cdot}2$). These values correspond to a duration of the effect of 133 and 850 min respectively.

The results indicate that the rate of fall in degree of impairment is correlated with the rate of fall in the blood ethanol concentration, since the slopes of both regression curves—impairment *vs* blood ethanol—were of the same order of magnitude, the differences not being significant ($P < 0{\cdot}2$) as shown by analysis of covariance. This result may be interpreted as one indication that the same mechanism, i.e. changes in ethanol concentration itself, governs the rate of change in cns impairment.

The difference in x-intercept, i.e. in threshold, was however statistically significant ($P < 0{\cdot}001$) when tested by analysis of covariance, inferring that the addition of pyrazole to ethanol brought about a significant lowering of the threshold (Goldberg 1943, 1966).

DISCUSSION

To elucidate the possible mechanism of action underlying the synergistic (or possibly supra-additive) effect observed for the two drugs the role played by three main processes must be considered:

(1) *Effect related to changes in course of the blood ethanol curve*

The difference in maximal impairment—8·7% after ethanol alone against 16·5% after ethanol + pyrazole—is greater than the difference in maximal blood ethanol concentration—1·33 against 1·61 mg ethanol/g blood. The main factor governing the peak effect is the maximal peak ethanol concentration, or rather the difference between peak concentration and threshold value, termed "active" peak concentration (Goldberg 1943, 1966), this was 0·81 (1·33–0·52) mg ethanol/ml blood in the ethanol condition and 1·61 (1·61–0) mg/ml in the ethanol + pyrazole condition, in good agreement with the actual peaks of impairment.

The duration of the impairment also increased from 130 min in the ethanol condition to 850 min in the ethanol + pyrazole condition, i.e. corresponding to the prolongation of the blood ethanol curve and the change in threshold value.

The rates of change in impairment with time also differed, being 0·084% per min and 0·023% per min respectively in the two conditions, corresponding to the rate of disappearance of ethanol from the blood, 0·0080 mg/ml per min against 0·0020 mg/ml per min. If changes in blood ethanol concentration were a major factor in governing the cns effects and the mechanisms of action in the ethanol and in the ethanol + pyrazole condition were similar, the ratio between various impairment parameters studied and the corresponding blood ethanol parameters would be the same in the two conditions. Actual ratio values found were 10·3 and 10·7 for maximal impairment *vs* blood ethanol maximum, 10·5 and 11·5 for rate of decline, 9·9 and 9·7 for area of impairment *vs* area of "active" blood ethanol concentration, and 9·7 and 11·1 for rate impairment *vs* rate of fall of blood ethanol concentration. The actual ratios found were all of the same order of magnitude thus in good agreement with the proposed mechanism.

(2) *Possible effects mediated via metabolites and metabolic changes*

With regard to the possibility that metabolic changes elicited the central nervous effects observed, such a mechanism is not very likely, as ethanol-induced secondary metabolic effects, e.g. the shift in the NAD^+: NADH ratio, or the change in the lactate: pyruvate ratio are partially restored to normal by the addition of pyrazole or its derivatives (Bustos & others, 1970; Blomstrand & Theorell, 1970).

The concentration of acetaldehyde was decreased when pyrazole was given together with ethanol (Lester & others 1968). In experiments with [1-^{14}C]ethanol, pyrazole induced a reduction in $^{14}CO_2$-formation of the same order of magnitude as the reduction in metabolic rate of ethanol (Goldberg & Rydberg, 1969). Thus, the ethanol-induced metabolic effects are partially counteracted by pyrazole, whilst we found the cns effects of ethanol to be increased by pyrazole. The cns effects observed are quantitatively accounted for by the changes in blood ethanol itself and by the cns effects of pyrazole.

Pyrazole thus can serve as an experimental tool in the study of ethanol-induced cns effects to differentiate between certain primary effects, and secondary, mainly metabolic, effects of ethanol.

(3) *Acute and long-term pyrazole-induced cns deterioration*

An acute cns-deteriorating effect was demonstrated by the gradually increasing cns impairment after pyrazole alone, being maximal 10 h after intake (Fig. 1A).

The long-lasting acute action points to a slow elimination of pyrazole, rather than to a non-reversible blocking action of pyrazole on LADH, as judged from the competitive, dose-dependent nature of the LADH-pyrazole interaction (Theorell & Yonetani 1963; Goldberg & Rydberg 1969). The shape of the curvilinear decline of the blood ethanol curve after addition of pyrazole may be interpreted as the consequence of the elimination of pyrazole being slower than that of ethanol (Fig. 1). Pilot experiments in rats (Rydberg, Buijten & Neri, 1972) have shown a half-life of pyrazole concentration in blood of 13 h. When ethanol also was given, the half life of pyrazole increased to 21 h, against about 2 h for the ethanol dose used. A LADH-inhibiting activity of pyrazole has been demonstrated to last for several days after a single administration (Goldberg & Rydberg, 1969).

As has been pointed out by Krebs (1970), the administration of pyrazole alone brings about an increase in ethanol concentration in the hepatic vein, indicating a reduction in the rate at which the endogenously formed ethanol—which can be demonstrated in the portal vein—is metabolized. Krebs (1970) found no ethanol in peripheral blood in his experiments. In the present study we did not detect any ethanol in peripheral blood, when only pyrazole was administered with saline. However, the performance of the animals was influenced by the administration of pyrazole alone, as manifested by the significant impairment increasing with time (Figs 1, 2). Another indication of an acute cns effect of pyrazole was the lowering of the threshold value induced by the addition of pyrazole to ethanol. This demonstrates the same trend as the findings of Blum, Geller & Wallace (1971) that both pyrazole and 3-methylpyrazole, a non-inhibitor of LADH, induced a deterioration when combined with ethanol. Furthermore, a long-term post-drug deterioration, lasting for one week or longer after a single administration of pyrazole, ethanol and ethanol + pyrazole, was observed; the maximum impairment of 5·6 % occurred at 7 h (Fig. 2).

No post-drug effects were observed in animals exposed to saline or ethanol only. The effects found may be looked upon as long-term toxic effects of exposure to pyrazole and ethanol + pyrazole.

The post-drug effects observed (Fig. 2) may be related to the long-term toxic effects of pyrazole affecting hepatic enzymes (Kiessling & Rydberg, 1969; Lelbach, 1969; Benson, 1970; Rubin & others, 1970; Lieber, Rubin & others, 1970).

Acknowledgements

We are indebted to J. Buijten and A. Bjursell for technical assistance, and to M. Rosenberg for computional work. The investigation has been defrayed by grants from the Swedish Medical Research Council to L. Goldberg (Project 14X-552) and to U. Rydberg (Project 25P-2903).

REFERENCES

ADMIRAND, W. H., CRONHOLM, T. & SJÖVALL, J. (1970). *Biochim. Biophys. Acta*, **202**, 343–348.
ARVOLA, A., SAMMALISTO, L. & WALLGREN, H. (1958). *Q. Jl Stud. Alc.*, **19**, 563–572.
BENSON, G. (1970). *Fedn Proc. Fedn Am. Soc. exp. Biol.*, **29**, 276.
BLOMSTRAND, R. & FORSSELL, L. (1971). *Life Sci. (II)*, **10**, 523-530.
BLOMSTRAND, R. & THEORELL, H. (1970). *Ibid.*, **9**, 631-640.
BLUM, K., GELLER, I. & WALLACE, J. E. (1971). *Br. J. Pharmac.*, **43**, 67–73.
BUSTOS, G. O., KALANT, H., KHANNA, J. M. & LOTH, J. (1970). *Science, N.Y.*, **168**, 1598–1599.
FELDSTEIN, A. & KURCHARSKI, J. M. (1971). *Life Sci., (I)*, **10**, 961–967.
GOLDBERG, J. M. & STRÖM, U. (1970). *Medicinsk riksstämma*, Stockholm, Abstracts, 304.

GOLDBERG, L. (1943). *Acta physiol. scand.*, **5**, *Suppl.* XVI, 128 pp.

GOLDBERG, L. (1966). *Psychosom. Med.*, **28**, 570–595.

GOLDBERG, L. & RYDBERG, U. (1966). In *Automation in Analytical Chemistry*, p. 595. Editor: Skeggs L. T. Jr. New York: Mediad Inc.

GOLDBERG, L. & RYDBERG, U. (1969). *Biochem. Pharmac.*, **18**, 1749–1762.

GOLDSTEIN, D. B. & PAL, N. (1971). *Science, N.Y.*, **172**, 288–290.

JOHNSON, O., HERNELL, O., FEX, G. & OLIVECRONA, T. (1971). *Life Sci. (II)*, **10**, 553–559.

KALANT, H. (1970). In *International Encyclopedia of Pharmacology and Therapeutics*, sect. 20, vol. I, p. 189. Editor: Tremolières, J. Oxford: Pergamon Press.

KALANT, H. & KHANNA, J. M. (1969). In *Biological and Clinical Aspects of Alcohol Metabolism*, p. 47. Editor: Sardesai, V. Springfield: C. C. Thomas.

KIESSLING, K. H. (1962). *Exptl. Cell Res.*, **26**, 432–434.

KIESSLING, K.-H. & RYDBERG, U. (1969). *Commun. Dept. Alc. Res. Karolinska Institutet*, 18. 06, 8 pp.

KREBS, H. A. (1970). *Biochem. J.*, **118**, 635–644.

KREBS, H. A., FREEDLAND, R. A., HEMS, R. & STUBBS, M. (1969). *Ibid.*, **112**, 117–124.

LELBACH, W. K. (1969). *Experientia*, **25**, 816–818.

LESTER, D., FELZENBERG, F. & KEOKOSKY, W. Z. (1968). *Q. Jl Stud. Alc.*, **29**, 449–454.

LIEBER, C. S., RUBIN, E., DE CARLI, L. M., MISRA, P. & GANG, H. (1970). *Lab. Invest.*, **22**, 615–621.

MORGAN, J. C. & DI LUZIO, N. R. (1970). *Proc. Soc. exp. Biol. Med.*, **134**, 462–466

REYNIER, M. (1969). *Acta chem. scand.*, **23**, 1119–1129.

REYNIER, M. (1970). *Agressologie*, **11**, 407–416.

RUBIN, E., GANG, H. & LIEBER, C. S. (1970). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **29**, 275.

RYDBERG, U., BUIJTEN, J. & NERI, A. (1972). *J. Pharm. Pharmac.*, **24**, 651–652.

THEORELL, H. (1965). *Experientia*, **21**, 553-561.

THEORELL, H. & YONETANI, T. (1963). *Biochem. Z.*, **338**, 637-653.

WALLGREN, H. (1966). *Psychosom. Med.*, **28**, 431–442.

WATKINS, W. D., GOODMAN, J. J. & TEPHLY, J. R. (1969). *Fedn Proc, Fedn Am. Socs. exp. Biol.*, **28** 546.

WIDMARK, E. M. P. (1930). *Les Lois Cardinales de la Distribution et du Metabolisme de l'Alcool Ethylique dans l'Organisme Humain* Kungl. Fysiografiska Sällskapets Handlingar, N.F. Bd **41**, 58 pp. Lund: Gleerups.