Brit. J. Pharmacol. (1957), **12**, 245.

THE RELATION BETWEEN SEDATION AND BODY TEMPERATURE IN THE MOUSE

BY

A. W. LESSIN AND M. W. PARKES

From the Biological Laboratories, Research Department, Roche Products Limited, Welwyn Garden City

(RECEIVED FEBRUARY 13, 1957)

Chlorpromazine and reserpine reduce locomotor activity and prolong pentobarbitone hypnosis in mice. Both these effects are shown to be proportional to the fall in body temperature produced by these drugs. Other agents are shown to reduce body temperature and potentiate pentobarbitone. At ambient temperatures of 32° C. neither chlorpromazine nor reserpine is hypothermic or sedative. It is concluded that sedative effects in the mouse at ordinary room temperatures are related to the hypothermic properties of these drugs. At 36° C., while reserpine fails to potentiate pentobarbitone, chlorpromazine still does so.

Sedation may be recognized as a condition of reduced activity which can be observed following the administration of hypnotics, such as the barbiturates, in sub-hypnotic doses. Chlorpromazine and reserpine are characterized by their ability to produce sedation, whilst being devoid of hypnotic properties. Both drugs potentiate the hypnotic effect of barbiturates in the mouse (Courvoisier, Fournel, Ducrot, Kolsky, and Koetschet, 1953; Kopera and Armitage, 1954; Cronheim and Toekes, 1954) and this property has been regarded as an expression of sedative action.

Lowered body temperature has been shown to prolong pentobarbitone anaesthesia (Fuhrman, 1947), and since chlorpromazine and reserpine cause hypothermia (Courvoisier *et al.*, 1953; Kopera and Armitage, 1954; Plummer, Earl, Schneider, Trapold, and Barrett, 1954) it seemed possible that these two properties were related. This possibility was supported by the evidence that various agents which lower body temperature, such as histamine, antihistamines, and pethidine (Packman, Rossi, and Harrisson, 1953; Dutta, 1948) also potentiate barbiturate anaesthesia (Winter, 1948; Ambrus, Ambrus, Leonard, Moser, and Harrisson, 1952; Kopera and Armitage, 1954).

In this paper we have investigated the relationship between body temperature and sedation, as measured by reduction in activity and potentiation of pentobarbitone hypnosis by sedative agents, in the mouse.

METHODS

Rectal temperatures were measured using a probe consisting of a type F thermistor mounted in a Perspex holder and registering directly on a microammeter in a circuit similar to that described by Grieve (1951). The probe was inserted 2 cm. into the rectum. Groups of 5 or 10 mice were used and the mean temperature recorded. At body temperatures of about 38° C., this method gave readings with a standard deviation ±0.6° C., increasing with lower temperatures, reaching ±1° C. at 35° C. and ±2° C. at 29° C.

Activity was measured by interruption of a beam of light, by a modification of the method of Dews (1953). Mice were placed singly in a Perspex counting chamber 13 cm. square for a period of 15 min., the scores being registered automatically. Rectal temperature readings were taken immediately before and after activity measurement and the mean recorded.

Sleeping time was taken as the period between injection of a mouse with pentobarbitone and the return of its ability to right itself when disturbed.

The room temperature was maintained at 22° C.; experiments at higher temperatures were performed in an incubator. Male albino mice of 16 to 24 g. weight were used. The mice were not starved before use; food and water were withheld during experiments lasting less than 6 hr. Pentobarbitone sodium was injected intravenously and all other drugs intraperitoneally, in volumes of 0.2 ml./20 g. All drugs were dissolved in saline with the exception of reserpine, which was dissolved in 2N acetic acid, neutralized as far as possible and diluted with water.

RESULTS

The Effect of Chlorpromazine and Reserpine on Body Temperature.—Chlorpromazine and reserpine caused a fall in rectal temperature of mice, which reached minimum levels 1½ to 3 hr. after injection of chlorpromazine (2 to 5 mg./kg.) (Fig. 1, B) and 4 to 8 hr. after reserpine (0.5 to 5 mg./kg.) (Fig. 2, B). Fig. 3 shows the minimum mean rectal temperature reached by groups of mice

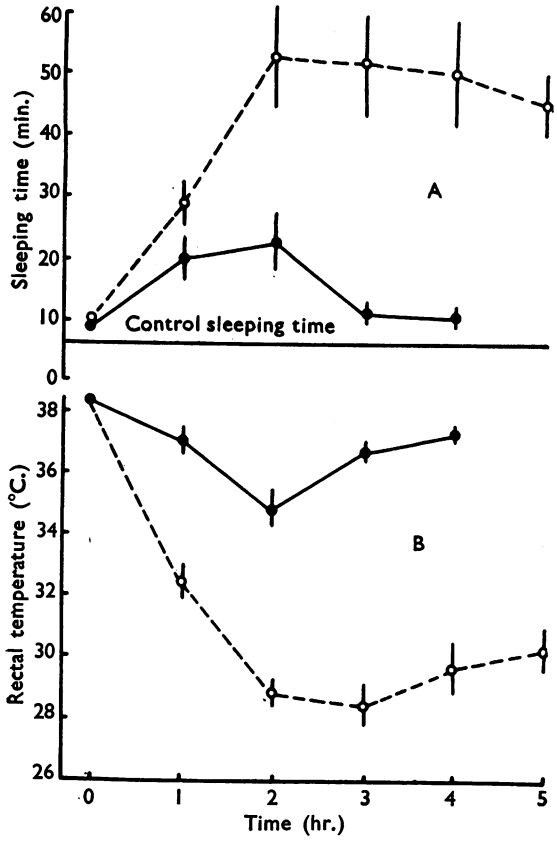

FIG. 1.—A. Effect of chlorpromazine 3 and 5 mg./kg. i.p., injected at 0, on the mean sleeping time of groups of 10 mice treated after various intervals with 25 mg./kg. pentobarbitone i.v. The sleeping time produced by the dose of pentobarbitone in normal mice is indicated. B. Mean rectal temperatures of these groups of mice taken immediately before injection of pentobarbitone. In both graphs, ●——●=3 and ○---○=5 mg./kg. chlorpromazine. The vertical lines represent standard errors of mean values. Room temperature, 22° C.

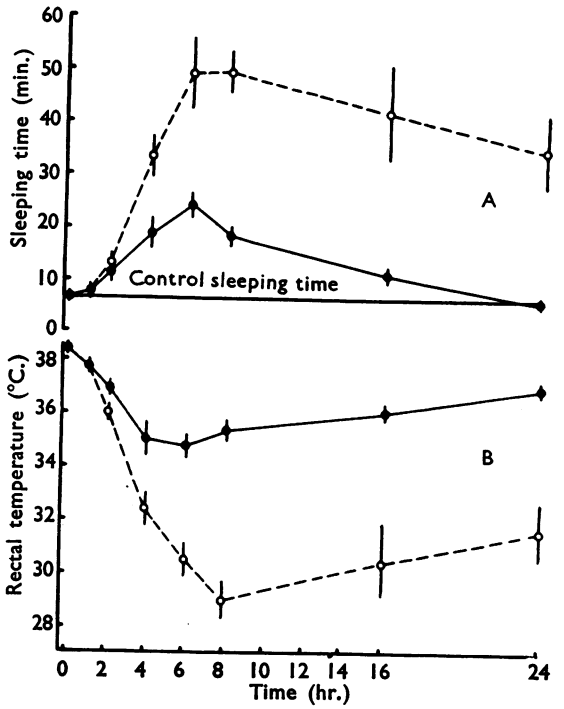

FIG. 2.—A. Effect of reserpine 1.0 and 1.5 mg./kg. i.p., injected at 0, on the mean sleeping time of groups of 10 mice treated after various intervals with 25 mg./kg. pentobarbitone i.v. The sleeping time produced by the dose of pentobarbitone in normal mice is indicated. B. Mean rectal temperatures of these groups of mice taken immediately before injection of pentobarbitone. In both graphs, ●——●=1.0 and ○---○=1.5 mg./kg. reserpine. The vertical lines represent standard errors of mean values. Room temperature, 22° C.

treated with chlorpromazine or reserpine, plotted against the logarithm of the dose. Temperatures are expressed as differences from controls.

There was a clear regression of temperature fall upon dose with each drug; no fall was produced by doses of less than 1.5 mg./kg. chlorpromazine or 0.3 mg./kg. reserpine.

Activity and Body Temperature.—Locomotor activity was measured in mice after injection of 0.25 to 2 mg./kg. chlorpromazine or reserpine. Measurements were made 30 min. to 2 hr. after chlorpromazine and 1 to 4 hr. after reserpine. The lowest doses were included to determine whether these drugs could reduce activity without lowering body temperature.

Activity counts varied from 10 to 160/15 min., with mean rectal temperatures of 35.0 to

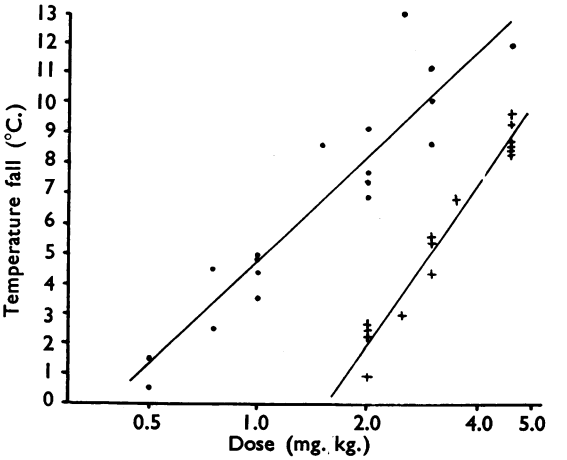

FIG. 3.—The effect of chlorpromazine and reserpine, given intraperitoneally, upon the rectal temperature of mice. Each value for temperature fall is the lowest recorded for a group of 5 mice after injection of the drug, and is expressed as the difference from the mean value for an untreated group. ●=Reserpine; b=12.0±1.9 (P=0.95); +=Chlorpromazine; b=17.8±2.2 (P=0.95).

39.0° C. A fall in rectal temperature to 34° C. was sufficient to abolish locomotor activity in the mouse. The temperature values were plotted against log. activity counts (Fig. 4) and correlation was apparent for each drug, as follows:

Chlorpromazine .. r=0.85 (73 readings)
Reserpine .. .. r=0.81 (56 readings)

Correlation was highly significant ($P<0.001$) in both cases. The regression lines did not differ significantly from each other in slope or position ($P>0.3$).

Untreated mice had rectal temperatures of 37.25 to 39.0° C. and activities of 50 to 170 counts per 15 min. There was little or no correlation (r=0.03), but it may be seen that the area enclosing the standard deviations of temperature and activity about the means for untreated mice was distributed about the upper ends of the regression lines for those treated with the drugs.

Potentiation of Pentobarbitone and Body Temperature.—The prolongation produced by 2 to 5 mg./kg. chlorpromazine and 0.5 to 3 mg./kg. reserpine upon the duration of pentobarbitone hypnosis increased with the interval between injection of the potentiating drug and the barbiturate (Figs. 1, A and 2, A), reaching a maximum 2 to 3 hr. after chlorpromazine and 6 to 8 hr. after reserpine.

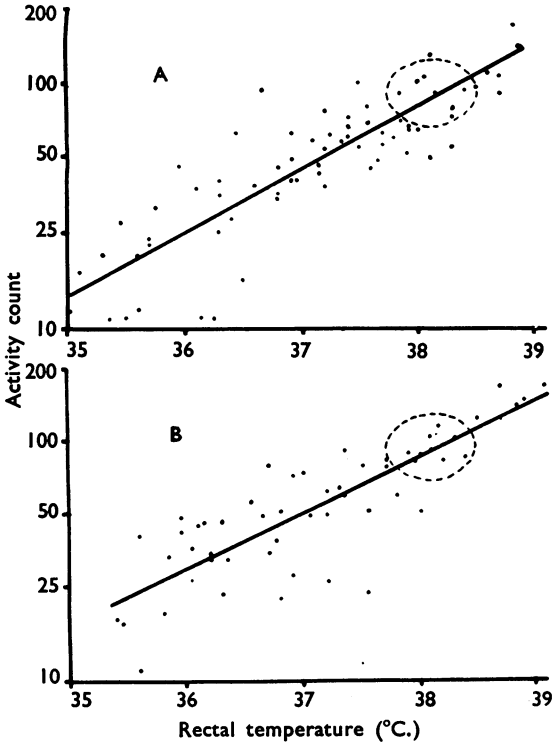

FIG. 4.—Rectal temperatures and activity counts for mice treated with (0.25 to 2 mg./kg.): A, chlorpromazine, and B, reserpine. Slope A=0.25±0.04 (P=0.95), 73 values; B=0.22±0.044 (P=0.95), 56 values. The area within the ellipse represents the standard deviations about the means of temperature and activity in 34 untreated mice. Room temperature, 22° C.

The time course for the hypothermia produced by each of these two drugs was followed, and Figs. 1, B and 2, B show that the curves for change in body temperature reflect the corresponding curves for barbiturate potentiation, minimum temperatures coinciding approximately with maximum prolongation of sleeping time.

A relation was clearly apparent between corresponding values for the two effects, and this was further examined, by using values for single mice. These were injected with 1.0 to 5.0 mg./kg. chlorpromazine or 0.5 to 3 mg./kg. reserpine, and, after the appropriate interval for maximum effect on body temperature, the rectal temperature was measured and 25 mg./kg. pentobarbitone injected. The resulting sleeping time for each mouse was plotted against its rectal temperature (Fig. 5). Significant correlation between temperature and subsequent sleeping time was found for both drugs (chlorpromazine −0.74, reserpine −0.9; $P<$ 0.001). The two regression lines could not be shown to differ significantly in slope or position, and both passed through values for temperature and sleeping time in mice treated only with pentobarbitone.

Among other substances found to lower rectal temperature and prolong pentobarbitone hypnosis were 5-hydroxytryptamine, for which potentiation of barbiturate has already been reported (Shore, Silver, and Brodie, 1955), adenosinetriphosphate, which is known to lower body temperature (Green and Stoner, 1950), and promethazine, for which both properties have been described (Courvoisier *et al.*, 1953; Packman *et al.*, 1953; Kopera and Armitage, 1954). Doses of these agents causing a similar decrease in temperature (Fig. 6) were found to prolong the sleeping time to the same extent (Table I). Intraperitoneal injections of saline, used in control experiments, were without effect on body temperature or pentobarbitone sleeping time.

Influence of External Temperature.—It has been shown that chlorpromazine in the rat (Maier, Forster, Schaff, and Kayser, 1955) and reserpine in the rabbit (Plummer *et al.*, 1954) do not reduce body temperature when the ambient temperature is about 31° C. (thermal neutrality), and this has been taken as evidence that these drugs lower body temperature by interfering with temperature regu-

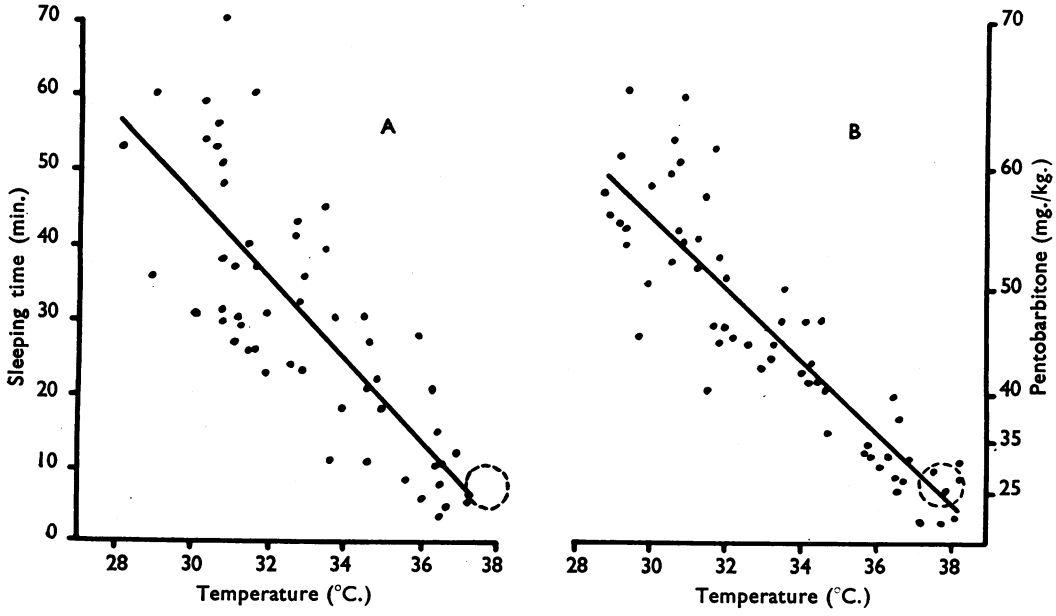

FIG. 5.—Rectal temperatures and sleeping times for mice treated with: A, 1.0 to 5.0 mg./kg. chlorpromazine, and B, 0.5 to 3 mg./kg. reserpine, followed by 25 mg./kg. pentobarbitone given 1 to 3 hr. after chlorpromazine and 4 to 6 hr. after reserpine. The temperature of each mouse was taken immediately before injecting the pentobarbitone. Room temperature, 22° C. Slope A= -5.32 ± 1.4 ($P=0.95$), 54 values; B= -4.91 ± 0.63 ($P=0.95$), 60 values. The area within the ellipse represents the standard deviations about the means of temperature and sleeping time in 20 mice treated only with 25 mg./kg. pentobarbitone. The sleeping times shown on the left-hand ordinate are produced in untreated mice by the doses of pentobarbitone shown on the right-hand ordinate.

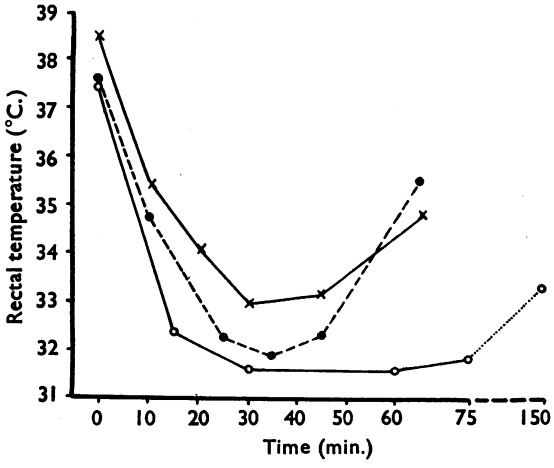

FIG. 6.—The effect of various agents on the mean rectal temperature of groups of mice. ×——×=5-hydroxytryptamine creatinine sulphate, 100 mg./kg. O——O=promethazine hydrochloride, 30 mg./kg. ●---●=adenosinetriphosphate, 200 mg./kg. Room temperature, 22° C.

lation. We have made similar observations in the mouse and have found that chlorpromazine and reserpine also produce no apparent sedation under these conditions. Both, however, prolong pentobarbitone anaesthesia at 32° C. (Table II). It was further found that pentobarbitone lowered body temperature at 32° C.; this effect was also prolonged by chlorpromazine and reserpine. At 36° C. the body temperature cannot fall and reserpine did not prolong pentobarbitone hypnosis at this temperature; chlorpromazine, however, exerted considerable potentiation (Table II).

TABLE I

THE EFFECT OF 5-HYDROXYTRYPTAMINE CREATININE SULPHATE, PROMETHAZINE AND ADENOSINETRIPHOSPHATE UPON THE SLEEPING TIME DUE TO 35 MG./KG. PENTOBARBITONE

Room temp. 22° C.

Pentobarbitone was injected 5 min. after the 5-hydroxytryptamine or adenosinetriphosphate and 45 min. after the promethazine. These intervals were chosen by reference to the period required for maximum effect of these drugs upon body temperature (Fig. 5).

	Sleeping Time (min.)±S.E.	
	Treated	Controls
5-Hydroxytryptamine, 100 mg./kg.	37·3±3·03	11·5±1·1
Promethazine, 30 mg./kg. ..	36·6±3·25	11·8±1·5
Adenosinetriphosphate, 200 mg./kg.	50.7±6·0	14·2±2·3

DISCUSSION

The results of activity measurement suggest a relationship between sedation and body temperature, since reduction in activity by both chlorpromazine and reserpine is proportional to the fall

TABLE II

THE DURATION OF PENTOBARBITONE HYPNOSIS IN MICE TREATED WITH RESERPINE OR CHLORPROMAZINE AT VARIOUS TEMPERATURES

Numerals in parenthesis denote the number of mice used. The sleeping times with chlorpromazine 3 mg./kg. all show significant potentiation ($P<0.001$). For reserpine 1 mg./kg., at 22° C. and 32° C., $P<0.001$, and at 36° C., $P\sim0.15$.

Room Temp. (°C.)	Pento-barbitone (mg./kg.) i.v.	Sleeping Time (min.)±S.E.		
		Control	2 Hr. after 1 mg./kg. Reserpine i.p.	1 Hr. after 3 mg./kg. Chlorproma-zine i.p.
22	25	7·9±0·9 (15)		24·0±2·4 (10)
22	35	11·8±1·5 (10)	26·3±2·9 (10)	
32	40	17·6±1·8 (10)	34·3±3·5 (10)	41·2±1·7 (10)
36	50	21·0±2·4 (10)	25·5±1·5 (10)	69·3±4·6 (10)

in rectal temperature which occurs. Moreover, the relationship appears to be the same for both these drugs. Neither chlorpromazine nor reserpine reduced activity significantly below the range found in untreated mice unless temperature was also reduced. It is therefore possible that these sedatives reduce activity in the mouse by virtue of their ability to lower body temperature.

This possibility is supported by the absence of apparent sedation in mice treated with chlorpromazine or reserpine at an ambient temperature of 32° C., at which these drugs caused no fall in body temperature.

A relationship has also been shown between the fall in temperature produced by the hypothermic drugs and their ability to prolong pentobarbitone hypnosis at room temperature. The correlation found between rectal temperature and sleeping time, and the similarity in this relationship for chlorpromazine and reserpine, suggests that their potentiation of hypnosis at normal environmental temperatures may be due to the fall in body temperature. In support of this, no evidence of potentiation could be found with doses of chlorpromazine or reserpine which failed to lower body temperature. Furthermore, a similar time course has been shown for both effects, minimum body temperature and maximum potentiation of pentobarbitone occurring together.

The variation in duration of barbiturate hypnosis with room temperature, reported by Raventós (1938) and others, may be explained by its dependence upon body temperature. Fuhrman (1947) related this to reduction by lower temperature of the activity of the enzyme responsible for detoxication, by showing that barbitone, which is largely excreted unchanged, was equally effective at different body temperatures. The dependence of detoxication rate upon temperature is also adequate to explain the prolongation of barbiturate hypnosis exerted by hypothermic agents at room temperature. Reference to Fig. 5 shows that a fall in body temperature of 10° C. increased sleeping time from 7 to 60 min. In terms of effective dose of pentobarbitone, taken from the dose-response curve, this represents the conversion of 25 mg./kg. to the equivalent of 65 mg./kg., that is, an increase by about 2.5-fold. This value is of the order which might be expected for the Q_{10} of a chemical reaction such as that responsible for the detoxication of pentobarbitone (Brodie, Burns, Mark, Lief, Bernstein, and Papper, 1953).

It may be suggested, therefore, that reserpine and chlorpromazine produce, by lowering body temperature, a reduction in the rate of detoxication. They would, on this supposition, have this mode of action in common with the potentiating agent β-diethylaminoethyl diphenylpropyl acetate (SKF 525A) (Cook, Toner, and Fellows, 1954), which, however, has been shown to inhibit the detoxicating enzyme system directly (Axelrod, Reichenthal, and Brodie, 1954) and shows no sedative action.

The results at 32° C. do not appear to bear out the relation between hypothermia and barbiturate potentiation suggested by the results at 22° C., for neither reserpine nor chlorpromazine are hypothermic at the higher temperature, whereas both prolonged pentobarbitone hypnosis. However, reserpine failed to potentiate the barbiturate at 36° C., at which no fall of body temperature can occur. In addition, it was found that pentobarbitone itself reduced the body temperature of mice at 32° C., and this effect was prolonged by reserpine. It may be concluded, therefore, that potentiation of barbiturate hypnosis by reserpine does not occur unless the body temperature falls.

This is certainly not true of chlorpromazine, which potentiated pentobarbitone hypnosis at both 32° C. and 36° C. At these temperatures, the action of this drug cannot be related to a hypothermic effect and represents an alternative means of potentiation which does not, however, appear to contribute to the potentiating action of chlorpromazine at lower room temperatures.

REFERENCES

Ambrus, J. L., Ambrus, C. M., Leonard, C. A., Moser, C. E., and Harrisson, J. W. E. (1952). *J. Amer. pharm. Ass.*, **41**, 606.

Axelrod, J., Reichenthal, J., and Brodie, B. B. (1954). *J. Pharmacol.*, **112**, 49.

Brodie, B. B., Burns, J. J., Mark, L. C., Lief, P. A., Bernstein, E., and Papper, E. M. (1953). Ibid., **109**, 26.

Cook, L., Toner, J. J., and Fellows, E. J. (1954). Ibid., **111**, 131.

Courvoisier, S., Fournel, J., Ducrot, R., Kolsky, M., and Koetschet, P. (1953). *Arch. int. Pharmacodyn.*, **92**, 305.

Cronheim, G., and Toekes, I. M. (1954). *Fed. Proc.*, **13**, 345.

Dews, P. B. (1953). *Brit. J. Pharmacol.*, **8**, 46.

Dutta, N. K. (1948). Ibid., **3**, 246.

Fuhrman, F. A. (1947). *Science*, **105**, 387.

Green, H. N., and Stoner, H. B. (1950). *Biological Actions of the Adenine Nucleotides.* London: H. K. Lewis.

Grieve, J. (1951). *Lancet*, **2**, 431.

Kopera, J., and Armitage, A. K. (1954). *Brit. J. Pharmacol.*, **9**, 392.

Maier, A., Forster, E., Schaff, G., and Kayser, Ch. (1955). *C.R. Soc. Biol., Paris*, **149**, 568.

Packman, E. W., Rossi, G. V., and Harrisson, J. W. E. (1953). *J. Pharm., Lond.*, **5**, 301.

Plummer, A. J., Earl, A., Schneider, J. A., Trapold, J., and Barrett, W. (1954). *Ann. N.Y. Acad. Sci.*, **59**, 8.

Raventós, J. (1938). *J. Pharmacol.*, **64**, 355.

Shore, P. A., Silver, S. L., and Brodie, B. B. (1955). *Experientia*, **11**, 272.

Winter, C. A. (1948). *J. Pharmacol.*, **94**, 7.