Biochemical Pharmacology, Vol. 19, pp. 3029–3037. Pergamon Press, 1970. Printed in Great Britain

EFFECT OF 2,4-DINITROPHENOL AND OTHER METABOLIC INHIBITORS ON THE RENAL DEPOSITION AND EXCRETION OF MERCURY*

T. W. CLARKSON and L. MAGOS

Department of Radiation Biology and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14620, U.S.A. and MRC Toxicology Unit, MRC Laboratories, Woodmansterne Road, Carshalton, Surrey, U.K.

Abstract—Observations are reported on the effects of administration of 2,4-dinitrophenol (DNP), probenecid, sodium fluoroacetate, sodium fluoride and sodium malonate on the ability of the rat kidney to accumulate inorganic mercury. Mercury was injected into female rats, body wt. 240 g, as mercuric chloride or mercuric cysteine (100 μg Hg per animal, i. m.) and the metabolic inhibitors were given subcutaneously in doses approaching the LD_{50} dose. Mercury levels were measured in urine, blood and kidney. DNP inhibited the renal accumulation of mercury. The other inhibitors were ineffective. DNP was effective only when given just prior to the injection of mercury. The dose of DNP capable of inhibiting renal uptake or mercury was without effect on renal hemodynamics and either did not influence, or only slightly influenced, the excretion of various urinary solutes and water. DNP did not increase the urinary excretion of mercury. It was concluded that the selective accumulation of mercury by kidney is because of energy-dependent processes and that most of this accumulation took place directly into renal tissue from the peritubular capillaries and not by way of glomerular filtration.

THE KIDNEYS play a central role in the pharmacokinetics of mercury. Approximately 50 per cent of the total excretion occurs via the renal route. This organ is the main site of deposition of mercury in the body. For example, some 2 weeks after a single injection of mercuric salts or an exposure to mercury vapor, over 70 per cent of the total body burden is found in the kidneys.[1,2] The same organ is also the main site of accumulation when rats are continuously exposed to vapor.[3] In general, the concentration of mercury in kidney tissue, expressed per gram wet weight, is fifty to one hundred times the concentration in almost all other tissues, and the concentration in other tissues is remarkably uniform.[3] If the ability of the kidneys to accumulate mercury is diminished, as for example by treatment with sodium maleate, the levels of mercury in other tissues rise.[4]

Very little is known about the mechanism underlying this selective renal accumulation. Reports on the pathways by which mercury enters the kidneys are conflicting. Some authors have presented evidence indicating that mercury is first filtered through the glomerulus prior to uptake by renal tissue[5–7] but contrary evidence has been reported by others.[8,9] Kidney uptake may proceed by transport of mercury directly from the peritubular capillaries to the renal cells.[10–12] It is perhaps not surprising that

* This paper is based, in part, on work performed under contract with the U.S. Atomic Energy Commission at the University of Rochester Atomic Energy Project and has been assigned Report No. UR-49-1126.

Dreisback and Taugner,[13] who utilized most of the various techniques used by others, concluded that mercury enters the tubular cells both through the brush borders and across the basal membrane.

Two possible mechanisms might be responsible for the selective accumulation of mercury by the kidneys. Mercury might attach to sites in kidney tissue possessing a uniquely high affinity for this metal. Alternatively, the renal cells may be able to utilize metabolic energy to actively accumulate mercury. With respect to the first possibility, it seems highly probable, on theoretical grounds, that mercury is bound to thiol groups.[14] There is experimental evidence implicating thiol-binding sites in kidney tissue.[15] Nevertheless, Clarkson and Magos[16] demonstrated that the thiol groups of the kidney have no higher affinity for mercury than thiol groups in other tissues. Thus, the concentrative uptake of mercury by kidney must require the expenditure of energy. In the case of uptake via glomerular filtration, the energy could be supplied by the concentrative uptake of water by the tubular cells. If selective accumulation were to take place from the peritubular capillaries, a specialized cellular metabolism must be involved such as obtains in the renal secretion of *para*-aminohippurate.

Clarkson and Magos[4] reported that sodium maleate, which is known to derange kidney metabolism, reduced the renal levels of mercury, but this was probably because of an accelerated release of the metal. This paper reports experiments with a variety of metabolic inhibitors with special reference to 2,4-dinitrophenol (DNP).

METHODS

White female (Porton or Rochester–Wistar strain) rats, 240 ± 10 g, were used. Food (MRC diet 41B or Purina Laboratory Chow) and water were available *ad lib.* The dose of mercury was always 100 μg Hg per animal given intramuscularly as mercuric chloride or mercuric cysteine. The preparation of these injection solutions, including the addition of the ^{203}Hg isotope, and the determinations of radioactive mercury in animal tissues and excreta were exactly as described previously.[4] The inhibitors were injected under the skin of the neck in 0·5 to 1·5 ml volumes. The following stock solutions were used for injecting the higher doses: (a) 0·6% 2,4-dinitrophenol (British Drug Houses, laboratory grade) in distilled water, containing sufficient sodium carbonate to completely dissolve the inhibitor; (b) 0·7% Probenecid (Benecid from Merck, Sharpe & Dohme, Ltd., Chemical name: *p*-(dipropylsylfamoyl)benzoic acid, in distilled water, complete solution being obtained by adding 2·0 N NaOH dropwise; (c) 0·05% sodium fluoroacetate (British Drug Houses, technical grade) in 0·9% NaCl; (d) 1·6% sodium fluoride (British Drug Houses, analytical grade) in distilled water; (e) 2·6% sodium malonate (British Drug Houses, laboratory grade) in distilled water. Suitable dilutions were prepared from the stock solutions in 0·9% NaCl.

Kidney function studies were carried out on animals housed in metabolic cages.[17] The animals were weighed and three placed in each cage. Food and water intake were calculated from the change in weight of the containers. The first 24-hr period in the cages was used to allow the animals to adjust to the new environment. More than the necessary number of cages was used, and at the end of this period the cages having similar urine volumes and food and water intakes were selected for the study. The urine was collected under toluene to prevent bacterial growth and to minimize changes

in pH. After collection, the urines were stored at 4° prior to analysis. When urine samples of known volume and composition were added to the metabolism cages over a 24-hr period and allowed to flow into the collectors, the following recoveries were obtained:[17] volume, 80 per cent, solutes, 90 per cent. At the end of the experiment, the animals were weighed, anesthetized with ether and a 5- to 10-ml vol. of blood removed from the aorta and transferred to a heparinized 15-ml conical centrifuge tube. Plasma was separated by centrifugation and the plasma samples frozen prior to analysis of endogenous creatinine and urea. The determination of urinary sodium, potassium, and chloride and of total urinary solutes by freezing point depression has been described previously.[17] Urea was measured by the microdiffusion method[18] and α-amino nitrogen and ammonia by the method of Clarkson and Ferraio.[19]

The analytical method used for the measurement of endogenous creatinine in plasma and urine, and the reasons for choosing creatinine clearance instead of inulin clearance as a measure of glomerular filtration rate, have been stated in detail in a previous publication.[20] To check that DNP did not interfere with the determination of endogenous creatinine, the determination was carried out on plasma and urine samples containing known amounts of DNP added *in vitro*. The maximum concentration of DNP tested corresponded to that concentration which would result if the entire dose to the animal were contained in the plasma volume or in the volume of an average urine sample. No differences were detected between treated and untreated samples.

RESULTS

A variety of metabolic inhibitors were screened by a rapid test to see if they affected the renal accumulation of mercury (Table 1). DNP was the only inhibitor which reduced renal uptake of mercury. The failure of the other inhibitors cannot be attributed to low doses. In most cases the maximum dose was close to the reported LD_{50} in rats. In the case of probenecid, such a high dose was not used since this inhibitor has been shown to produce 90–100 per cent inhibition of renal secretory systems at a dose

TABLE 1. COMPARISON OF THE EFFECTS OF VARIOUS METABOLIC INHIBITORS OF THE RENAL ACCUMULATION OF MERCURY*

Inhibitor	LD_{50} (mg/kg)	Ref.	Dose (mg/kg)	No.	Mercury in kidneys (%)	Significance
Sodium fluoroacetate	1·5	21	0·5	6	97·67 (± 3·45)	N.S.
Dinitrophenol	30	22	20·0	6	64·78 (± 2·47)	$P < 0.001$
Probenecid	611	23	30·0	6	99·25 (± 8·24)	N.S.
Probenecid	611	23	40·0	6	93·83 (± 3·78)	N.S.
Sodium fluoride	125 250	22	40·0	6	97·83 (± 6·58)	N.S.
Sodium malonate	2500	24	500·6	6	102·08 (± 8·39)	N.S.
Sodium malonate	2500	24	1000·0	6	92·42 (± 4·70)	N.S.

* The inhibitors were given 90 min prior to injection of mercuric cysteine, and the amount of mercury accumulated in the kidneys was measured 3 hr after injection of mercury. Mercury levels in treated animals are expressed as percentage of the paired controls. The absolute mean value for the mercury concentration in the control kidneys (18 animals) was 36·6 μg/g wet wt. The numbers in parenthesis are the standard errors of the means.

of 15 mg/kg.[25] The references quoted above and in Table 1 also indicate that all the inhibitors produce their characteristic effects within the $4\frac{1}{2}$-hr time period allowed for in the test. However, these results do not exclude the possibility that metabolic inhibitors, other than DNP may influence renal accumulation of mercury under different experimental conditions.

As a result of the findings in Table 1, further studies were made to explore the effect of DNP. As the dose of DNP was increased, the kidney level of mercury decreased (Fig. 1). The mercury level in the blood was higher in DNP-treated animals and the mercury in liver showed a dose-dependent increase.

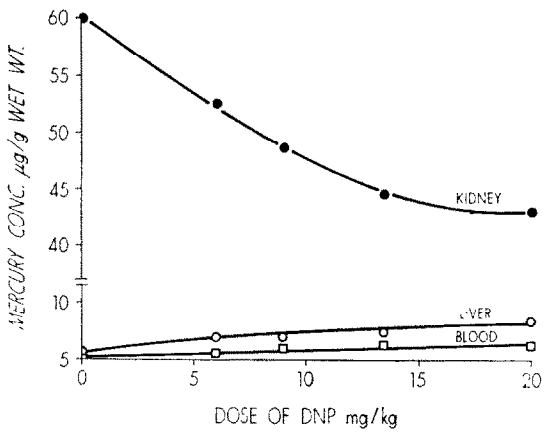

FIG. 1. Effect of increasing doses of DNP on the concentration of mercury in kidney, —●——●—; liver, —○——○—; and blood, —□——□—.

The decrease in the kidney level of mercury caused by DNP was not the result of an increased urinary excretion. This is demonstrated by the data in Table 2 describing the kidney level of mercury 24 hr after the administration of mercury and the 24-hr urinary excretion. It may be seen that DNP produced a net loss in the sum of the kidney and urinary mercury. This effect is evident whether mercury was injected as the mercuric cysteine salt or as mercuric chloride, despite the fact that the rates of urinary excretion and kidney uptake of mercury differ markedly for the two salts.

The possibility was tested that DNP, like sodium maleate, might influence the binding of mercury to the kidney and so enhance the release of mercury. DNP was given 2 and 12 days after injection of mercury and the 24-hr urinary excretion and the kidney level 24 hr after DNP administration were compared to renal excretion and deposition in control animals. As the data in Table 2 show, DNP given days after mercury had no effect on the kidney content of mercury.

A dose of DNP, 13·5 mg/kg, capable of reducing the renal deposition of mercury was without effect on endogenous creatinine clearance (Table 3). This finding is in agreement with observations of Mudge and Taggart[26] who reported that DNP can reduce the tubular transport of *para*-aminohippurate, diodrast or phenol red without affecting renal hemodynamics. This same dose of DNP is also without effect on the urea and endogenous creatinine clearances in mercury-treated animals (Table 4).

TABLE 2. EFFECT OF 2,4-DINITROPHENOL (DNP) ON THE KIDNEY CONTENT AND URINARY EXCRETION OF MERCURY*

Hg given as	DNP (mg/kg)	Urine coll. after Hg	No.	Kidney content of mercury		24-hr urinary excretion of Hg in per cent dose (b)	a + b
				Compared with paired controls	As per cent dose (a)		
$Hg(cyst)_2$	None	1st day	6	1·0	58·6	8·8	67·4
$Hg(cyst)_2$	13·5	1st day	6	0·67 (0·054)	38·4	9·2	47·6
$HgCl_2$	None	1st day	6	1·0	33·7	1·3	35·0
$HgCl_2$	13·5	1st day	6	0·78 (0·050)	25·2	1·4	26·6
$HgCl_2$	None	3rd day	2	1·0	49·2	2·4	51·6
$HgCl_2$	13·5	3rd day	2	0·94	46·0	2·7	48·7
$HgCl_2$	None	13th day	6	1·0	42·1	0·5	42·0
$HgCl_2$	13·5	13th day	6	1·04 (0·034)	43·7	0·6	44·3

* DNP was given 90 min before mercuric cysteine. The animals treated with $HgCl_2$ were given the same dose of DNP just before the mercury or 2 and 12 days later. The animals were placed in pairs in metabolism cages and the urine was collected during the first 24 hr after the DNP injection. At the end of this period, the animals were killed and the kidney content of mercury was measured. The numbers in brackets are the standard errors.

TABLE 3. EFFECT OF 13·5 mg/kg OF 2,4-DINITROPHENOL (DNP) ON ENDOGENOUS CREATININE CLEARANCE*

Treatment	No. of clearance estimations	Endogenous creatinine clearance (ml/min kg)	
		Before	After
Saline	6	7·4 (0·4)	7·6 (0·3)
DNP	6	7·7 (0·08)	7·2 (0·6)

* Urinary excretion of creatinine was measured in the 24-hr period before and after the administration of DNP or saline. Plasma levels were measured at the end of the experiment. The saline was given in a volume equal to that of the DNP injection solution. The numbers in parentheses are standard errors.

DNP at 13·5 mg/kg produced a significant increase in the excretion of water, chloride ions and urea plus ammonium ions and a barely significant increase in the excretion of total solutes (Table 5). The conclusion with respect to the changes in excretion of ammonium and urea is doubtful because the excretion rate in the second control period (11·9 m-equiv./kg) was considerably less than in the first (19·7 m-equiv./kg). The excretion rates of sodium and potassium were unaffected. These changes in excretion are small and would represent no more than a 1 per cent change in the absorptive efficiency of the renal tubules.[28] These results are in agreement with the findings of other investigators.[26,29,30]

TABLE 4. EFFECT OF 13·5 mg/kg OF 2,4-DINITROPHENOL (DNP) ON THE UREA AND ENDOGENOUS CREATININE CLEARANCES IN RATS GIVEN $HgCl_2$*

Urine collection after treatment (Hr)	No. of estimations	Clearances				Mercury in kidneys (Per cent dose)
		Creatinine		Urea		
		Before	After	Before	After	
		(ml/min kg)				
0–4	3	5·4	6·9	1·2	1·9	5·6
0–12	3	8·8	6·4	1·7	1·2	9·8
0–24	3	8·0	7·0	1·2	1·0	13·4
0–24 (Controls)	3					26·0

* Urinary excretion of urea and creatinine was measured in the 24-hr period before dosing with DNP and $HgCl_2$ and over various periods after dosing as indicated in the table. Plasma levels of creatinine and kidney levels of mercury were measured at the end of the experiment. The control animals were given an equal volume of saline.

DNP at the same dose increased the urinary excretion of α-amino-nitrogen. The mean α-amino-nitrogen excretion prior to dosing with DNP was 0·33 m-moles/kg in 24 hr from five metabolic cages, each containing three rats. In the 24-hr period after 13·5 mg/kg of DNP, the mean excretion was 0·49. The difference was significant ($P = 0·01$).

DISCUSSION

The absence of any effect of probenecid, sodium fluoroacetate, and malonate, which all inhibit the tubular secretion of *para*-aminohippurate *in vivo* or *in vitro*,[31] shows that the renal uptake of inorganic mercury is not linked to the organic acid transport system. The same conclusion was drawn by Campbell[32] and Driesback and Taugner[13] from studies with probenecid. Moreover, the lack of effect of sodium fluoroacetate and malonate indicates that the Krebs cycle is not crucial for the renal transport of mercury.

The lack of effect of probenecid on the renal excretion and accumulation of inorganic mercury contrasts with its reported inhibitory action on the uptake and excretion of organomercurial diuretics.[32–34] These diuretics, with the exception of chlormerodin, are probably secreted via the *para*-aminohippurate pathway.[35] The uptake of chlormerodin by the canine kidney is also inhibited by probenecid.[34] In this case it was suggested that the agent was inhibiting the uptake of the mercurial from the glomerular filtrate. However, it should be noted that all the published studies on the effects of probenecid on the renal uptake of organo-mercurials were carried out in different species than the one used in our experiments.

Cafruny and Ross[36] reported in an abstract that DNP did not affect renal accumulation of mercury in rats injected with mercuric chloride. Until more details are available it is not possible to discuss the reasons for the discrepancy with our results, except to say that it may be related to difference in doses and methods of administration.

In view of these negative findings, the specific effect of DNP is all the more interesting DNP affects the only uptake process since it is unable to produce any release of mercury from the kidney. It is known that DNP given in the same dosage range as used in the present experiments acts as an uncoupling agent in rats.[37] Thus, these

TABLE 5. THE EFFECT OF 2,4-DINITROPHENOL (DNP) (13·5 mg/kg) ON URINARY EXCRETION*

Cage	Rates of urinary excretion												
	24 hr prior to injection							24 hr after injection					
	Av. body wt. (g)	Vol. (ml/kg)	Cl^-	Na^+ (m-equiv./kg)	K^+	NH^+_4 and urea	Solutes (F.P.) (m-osmoles/kg)	Vol. (ml/kg)	Cl^-	Na^+ (m-equiv./kg)	K^+	NH^+_4 and urea	Solutes (F.P.) (m-osmoles/kg)
Controls	222	48·6	7·70	14·1	20·1	19·7	89·5	48·4	8·6	15·1	20·5	11·9	82·5
Experimentals	223	43·5	5·83	11·5	19·3	16·0	78·4	75·3	10·3	11·2	19·1	24·3	94
Statistic, P								0·01	0·04	>0·1	>0·1	0·02	0·05

* Each figure is the mean excretion from six metabolism cages housing three rats. P values were computed by the non-parametric statistic of Mann–Whitney, as cited by Auble.[27]

studies with DNP indicate that at least part of the renal uptake of mercury requires metabolic energy and that this energy is supplied by the energy-rich phosphates. As DNP caused a 34 per cent decrease in the kidney mercury level of animals dosed with mercuric cysteine and a 26 per cent decrease when animals were given mercuric chloride, it might be suggested that the metabolically-dependent uptake contributed at least 26–34 per cent to the total kidney uptake. These figures are minimal since it is unlikely that DNP produced a complete block of ATP synthesis.

It is possible that DNP might depress renal accumulation of mercury by mechanisms unrelated to its effect on oxidative phosphorylation. However, our experimental results eliminate the following alternative mechanisms. First, the uptake of mercury could be affected by changes in renal hemodynamics. However, the urea and endogenous creatinine clearances were unchanged. Furthermore, DNP did not cause gross disturbances in other renal functions—the urinary excretion rates of a variety of solutes and water were either unchanged or only slightly affected by DNP. Second, reduced renal uptake of mercury could be secondary to lower blood levels. For example, DNP might accelerate uptake by nonrenal tissue or enhance excretion. However, the facts are against this possibility. Blood levels are slightly increased after DNP and urinary excretion is unchanged. It is reported that DNP, in the same doses as used in the present experiments, inhibits bile flow in rats.* The small increase in liver concentrations of mercury is probably because of the blockage of bile flow and the slightly elevated blood levels. Third, it has been recently demonstrated that DNP may act as a competitive inhibitor of the *para*-aminohippurate excretory mechanism.[38] However, it is unlikely that DNP influences mercury uptake in this manner because probenecid is without effect.

The reduction in renal accumulation of mercury produced by DNP was not accompanied by an increase in urinary excretion. This observation is not compatible with the hypothesis that mercury enters the kidney by glomerular filtration followed by uptake into renal tissue. In this case inhibition of uptake by DNP would result in an increase in urinary excretion equivalent to the observed fall in kidney levels. Our results are compatible with the alternative pathway into kidney tissue; viz., directly from the peritubular capillaries.

Acknowledgements—We wish to thank Dr. J. M. Barnes for his critical reading of the manuscript, and Mr. L. Ferraio and Mr. R. Lock for excellent technical assistance.

REFERENCES

1. A. ROTHSTEIN and A. D. HAYES, *J. Pharmac. exp. Ther.* **130,** 166 (1960).
2. A. D. HAYES and A. ROTHSTEIN, *J. Pharmac. exp. Ther.* **138,** 1 (1962).
3. A. ROTHSTEIN and A. HAYES, *Hlth Physics* **10,** 1099 (1964).
4. T. W. CLARKSON and L. MAGOS, *Br. J. Pharmac. Chemother.* **31,** 560 (1967).
5. A. E. RODIN and C. N. CROWSON, *Am. J. Path.* **41,** 297 (1962).
6. W. WOCKEL, H. F. STEGNER and W. JANISCH, *Virchows Arch. path. Anat.* **334,** 503 (1961).
7. M. STAEMLER, *Virchows Arch. path. Anat.* **328,** 1 (1956).
8. M. BERLIN and S. GIBSON, *Archs envir. Hlth* **6,** 617 (1967).
9. J. GAYER and R. PARTOWI, *Ztschr f.d.g. exp. Med.* **135,** 419 (1962).
10. A. BERGSTRAND, L. FRIBERG, L. MENDEL and G. OBELBLAD, *J. Ultrastruct. Res.* **3,** 328 (1959).
11. A. M. MAMBOURG and C. RAYNAUD, *Rev. fr. Edud. Clin. biol.* **10,** 414 (1965).
12. J. VOSTAL, *Proc. 15th Int. Cong. Occup. Hlth, Vienna* **3,** 67 (1966).

* J. MacGregor, personal communication.

13. R. H. Dreisback and R. Taugner, *Nuclear Med.* **5**, 421 (1966).
14. H. Passow, A. Rothstein and T. W. Clarkson, *Pharmac. Rev.* **13**, 185 (1961).
15. J. Gayer, E. H. Graul and H. Hundeshagen, *Klin. Wschr.* **40**, 953 (1962).
16. T. W. Clarkson and L. Magos, *Biochem. J.* **99**, 62 (1966).
17. T. W. Clarkson and M. Greenwood, *Br. J. Pharmac. Chemother.* **26**, 50 (1966).
18. E. J. Conway, in *Microdiffusion Analysis and Volumetric Error*, p. 162. Chemical Publishing Co., New York (1963).
19. T. W. Clarkson and L. Ferraio, *Clin. Chem.* **14**, 433 (1969).
20. T. Stoytchey, L. Magos and T. W. Clarkson, *Europ. J. Pharmac.* **9**, 253 (1969).
21. A. Linderbaum, M. R. White and J. Schubert, *J. biol. Chem.* **190**, 585 (1951).
22. The Merck Index, p. 376. Merck & Co., Inc., Rahway, New Jersey.
23. S. E. McKinney, H. M. Peck, J. B. Bochey, B. B. Byan, G. S. Shuchurdt and K. H. Beyer, *J. Pharmac. exp. Ther.* **102**, 208 (1951).
24. C. M. Gruber, C. de Bernardinis and L. A. Erf, *Archs int. Pharmacodyn. Ther.* **79**, 461 (1949).
25. B. Rennick and A. Farah, *J. Pharmac. exp. Ther.* **116**, 287 (1956).
26. G. H. Mudge and J. V. Taggart, *Am. J. Physiol.* **161**, 173 (1950).
27. D. Auble, *Bull. Inst. educ. Res. Indiana Univ.* **1**, 1 (1953).
28. H. W. Smith, in *Principles of Renal Physiology*, p. 98. Oxford.
29. J. C. Strickler and R. H. Kessler, *Am. J. Physiol.* **205**, 117 (1963).
30. R. J. Chertox, W. H. Hulet and B. Epstein, *Am. J. Physiol.* **211**, 1379 (1966).
31. H. C. Hodge and F. A. Smith, in *Fluorine Chemistry* (Ed. J. H. Simons), Vol. IV, p. 202, Academic Press, New York (1965).
32. D. E. S. Campbell, *Acta pharmac. Tox.* **16**, 151 (1959).
33. J. M. Weiner, A. E. Burnett and B. R. Rennick, *J. Pharmac. exp. Ther.* **118**, 470 (1956).
34. E. J. Cafruny, K. C. Cho and R. Z. Gussin, *Ann. N. Y. Acad. Sci.* **139**, 362 (1966).
35. E. J. Cafruny, *Pharmac. Rev.* **20**, 89 (1968).
36. E. J. Cafruny and C. Ross, *Fedn Proc.* **19**, 361 (1960).
37. M. A. M. Cameron, *Br. J. Pharmac. Chemother.* **13**, 25 (1958).
38. W. O. Berndt and D. Grote, *J. Pharmac. exp. Ther.* **164**, 223 (1968).