Brit. J. Pharmacol. (1956), **11**, 209.

DISTRIBUTION OF MEPACRINE IN THE ORGANS OF DIFFERENT ANIMAL SPECIES, AND IN THE COMPONENTS OF LIVER CELLS

BY

G. A. SNOW AND E. WESTON HURST

From Imperial Chemical (Pharmaceuticals) Limited, Hexagon House, Blackley, Manchester, 9

(RECEIVED FEBRUARY 16, 1956)

In a previous paper (Hurst, Snow, and Roberts, 1955) it was shown that the marked therapeutic effect of mepacrine dihydrochloride dihydrate (mepacrine) against equine encephalomyelitis in the mouse (Hurst, Melvin, and Peters, 1952; Hurst, Peters, and Melvin, 1952) could not be reproduced in a number of other animal species. The drug appeared to have no effect, or even an adverse effect, on infections in chicks, guinea-pigs, rabbits, and monkeys. Only in the adolescent rat could a significant protective action be demonstrated. Mepacrine failed to protect newly weaned rats from infection, whereas it had a good therapeutic action on similarly immature mice.

Since mepacrine is quite exceptional in showing a therapeutic action against some smaller viruses, it seemed important to try to discover why its action should be largely confined to the mouse. Various reasons for this selective action could be imagined. There might, for instance, be a difference in metabolism in the mouse leading to the production of an active substance not formed in other species. The importance of metabolism in the action of mepacrine is still an unsettled question. On the one hand there is some biological evidence favouring the possibility that mepacrine may exhibit its antiviral activity because of conversion *in vivo* into an active product; on the other hand there is the observation (Goodall, 1956) that the acridines extractable from livers of mepacrine-treated mice contain at least 96% of unchanged mepacrine either free or in the form of salts with phospholipids. Another possible way in which the mouse could differ from other species is in the mode of distribution of mepacrine, either in the different tissues of the body or in different cellular components. It is this possibility which has been examined in the experiments described here. Although scattered references to the concentrations of mepacrine in various animal tissues occur in the literature, it has not been possible to make use of them. Most of them, e.g. Hecht (1936), Oldham and Kelsey (1945), Dearborn (1947), refer to small single doses of mepacrine or to small amounts administered daily over a period, whereas others give results which are insufficient for any comparisons to be made (e.g. Scudi and Hamlin, 1943). A systematic comparison of the levels of mepacrine in organs of five different animal species has therefore been made at various times after oral administration of a single massive dose of the drug.

Another line of approach was suggested by the observation (Hurst *et al.*, 1955) that liver cells of mice treated with mepacrine contain yellow cytoplasmic particles which fluoresce and which may be stained with basic dyes; certain other species such as the guinea-pig do not exhibit this effect. It was thought that the quantitative distribution of mepacrine in the different component parts of the liver cell might be important in relation to the antiviral action of the drug. Experiments involving the centrifugal separation of cellular particles were therefore performed with livers from mice and guinea-pigs previously treated with mepacrine.

METHODS

Animals

The animals used and their weight ranges were as follows (dose of mepacrine in mg./kg. in parentheses): mouse, 20–25 g. (500); rat, 230–300 g. (500); guinea-pig, 200–250 g. (200); rabbit, about 2,000 g. (300); chick, 40–50 g. (400). Mepacrine was given as a single oral dose, guinea-pigs being dosed with a fine catheter. Animals were decapitated or stunned and exsanguinated; organs for mepacrine determination were stored at −10°; livers for separation of cell components were homogenized at once, or, where the period after treatment was 6 or 12 hr., were stored overnight at 2°.

Determination of Mepacrine in Tissues

The weighed tissue was homogenized in water (10 ml./g. tissue) in a Waring blender. While the machine was

still running quickly enough to keep the suspension homogeneous a portion (1 or 2 ml.) was removed and shaken for 30 sec. with butanol (4 ml.). After centrifugation the solvent layer was removed and the aqueous layer and tissue extracted further with butanol (2 ml./extraction). A total of three butanol extractions usually sufficed, but occasionally a fourth was needed. The combined butanol extracts were mixed with ether (20 ml.) and extracted twice with 0.1 N-HCl (5 ml. and 3 ml.). The combined acid extract was diluted to 10 ml. and the optical density measured at 430 mμ on a Beckman spectrophotometer (1–4 cm. cell according to density). The result was converted to μg. of the mepacrine salt/g. moist tissue taking the extinction value 9,100 for the mepacrine absorption.

The procedure gave a quantitative extraction for all tissues except chick liver, where the yellow acridine-like material in the butanol extract could not be completely extracted into hydrochloric acid. The numbers given in the table refer only to acid-extractable material. The non-extractable product, which was present in concentration comparable to that of the mepacrine, gave an absorption spectrum in *iso*propanol differing widely from that of mepacrine, and showing maxima at 452 and 470 mμ.

Separation of Cell Components and Determination of Acridine Contents

The homogenization of the livers and separation of cell components were carried out at 0–5° with sucrose solution as the suspending medium. For convenience the tissue pulp was divided; one part was used for separation of nuclei and the other for the smaller cell components. Details are given in Table I.

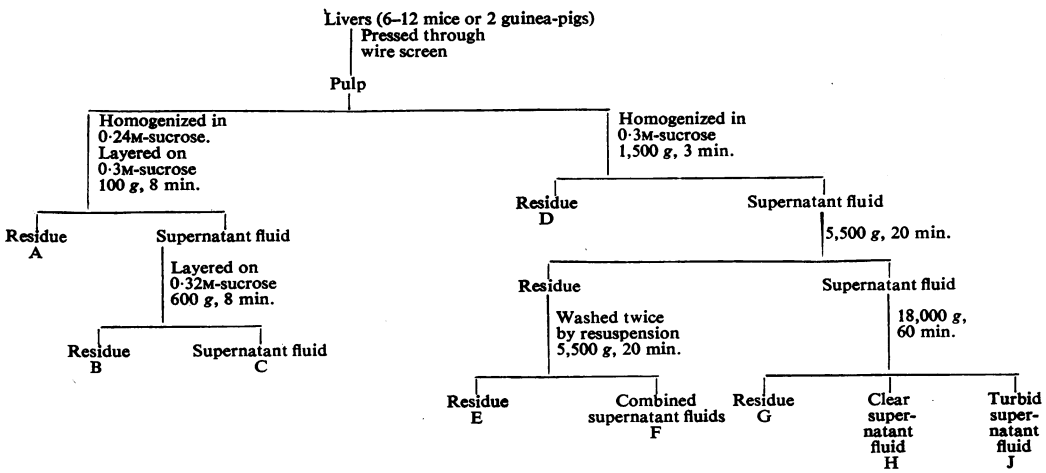
Total acridine was determined in all component suspensions (except J) by the method used for tissues. A check on recovery was made by totalling the contents estimated for fractions A–C and those for fractions D–H. A difference of not more than 10% between these totals was regarded as tolerable having regard to the possible summation of errors on a large number of estimations and the inevitable mechanical losses in the operations. The known loss in fraction J was usually negligible. The calculation of the actual acridine content of the different cellular components from the measured contents of the fractions separated experimentally required some consideration of the cellular composition of these fractions. It was possible to arrive at fairly reliable figures by utilizing the fact that the concentration in the mitochondrial particles was always very much higher than in the microsomal particles or the cell plasma. In discussing the method of calculation, the different cellular components will be considered in turn.

Nuclear Component.—Fraction A, which was nominally cellular debris, contained considerable quantities of nuclei. Fraction B contained little but nuclei whereas there were only occasional nuclei to be seen in fraction C. The acridine content of fraction A and B was usually of the same order. Since there were so many nuclei in A the nuclear component was taken as the sum of A+B. The mepacrine values for the nuclear component are thus rather too high, since they include any acridine contained in connective tissues and in a few unbroken cells.

Mitochondrial Component.—The mitochondrial particles were present in fractions D, E, and F. In D were found the largest of the mitochondrial particles accompanied by the whole of the nuclei and debris; E contained little but mitochondrial particles whereas F contained mitochondrial particles with perhaps 15–20% of the total cell plasma and microsomes. The latter made

TABLE I

SEPARATION OF CELL COMPONENTS FROM LIVERS OF MOUSE OR GUINEA-PIG



Figures indicate centrifugal acceleration and time.
* Part of supernatant fluid is always turbid because of unavoidable disturbance of the residue in removal of the last drops.

only a small contribution to the acridine content and the slight excess due to this source was left uncorrected to offset partially the unavoidable mechanical losses. The total acridine in the mitochondrial fraction was therefore calculated as D+E+F−(A+B). (Sum of acridine in all mitochondria-containing fractions less the acridine known to be present in the debris and nuclei.)

Microsomal Component.—Consideration of the volumes of extracts and precipitates at various stages of the separation showed that the microsomal component must be widely distributed in different fractions and fraction G can only represent a part of the total. Microsomes are lost in fractions D, F, and J. It was estimated that recovery of microsomes in G was of the order of 50%. For the purposes of these calculations therefore, the mepacrine content of fraction G has been doubled to give a figure for the content of the microsomal fraction. This is admittedly only a crude approximation and the mepacrine values for this component must be regarded as rather inaccurate.

Cell Plasma.—A fairly exact determination of the content of cell plasma could be made. Since the original preparation involved the homogenization of liver with sucrose solution in the ratio 5 ml. sucrose/g. liver, the volume of plasma+sucrose solution in the suspension could reasonably be taken as 5.32 ml./g. liver. The final clear supernatant fluid (H) after removal of all the particulate cell components could be regarded as an aliquot of this solution and hence the acridine content of cell plasma determined.

Effect of Time of Dosing on the Therapeutic Response to Mepacrine in the Mouse and Rat

Newly weaned mice or young rats (30–35 g.) in groups of 20 were used. They received a single dose of mepacrine hydrochloride (350 mg./kg.) at times varying from 5 days before to 1 day after intramuscular infection with Eastern equine encephalomyelitis. Virus strains and conditions of experiment were as in previous investigations (see Hurst *et al.*, 1952). Mortalities and times of survival are given in Table III.

RESULTS

Mepacrine Levels in the Organs of Different Species

The species chosen for comparison were mouse, rat, guinea-pig, rabbit, and chick. In order to make the experiments as nearly comparable as possible the animals were all given a single large oral dose. The amount chosen was the largest that could be given without causing evident toxic effects. Hence the doses were not identical on a weight basis, but varied by a factor of 2.5 from the highest (rat and mouse) to the lowest (guinea-pig). Various methods have been described for the extraction of mepacrine from tissues. In the older methods (Hecht, 1936; Dearborn, Kelsey, Oldham, and Geiling, 1943) the tissue proteins were denatured by heating with alkali or acid, but more recently the direct extraction method of Brodie and Udenfriend (1943) has been favoured. This involves the use of ethylene dichloride. In the present experiments butanol has been used as the extracting solvent. Ether was added to the butanolic extract and the acridine transferred to aqueous solution as hydrochloride; the optical density was measured at 430 mμ, which is near a peak in the mepacrine absorption spectrum. The results were calculated as mepacrine but represent the total acridine extractable under these conditions (assuming a comparable absorption at 430 mμ). In mouse liver it has been shown (Goodall, 1956) that at least 96% of the extractable acridine is mepacrine. The composition in other tissues is unknown, but absorption measurements above and below 430 mμ showed that there was a true peak at this wavelength, which is probably characteristic of the acridine nucleus present in mepacrine. Metabolic change in the side chain would not be revealed as a change of spectrum unless the whole side chain were lost. The only anomaly in this extraction procedure was found with chick liver, where it was possible to extract only a part of the acridine from the organic solvent phase into 0.1 N-HCl. Examination of the yellow material remaining in the organic solvent layer showed that it was not a mepacrine complex with phospholipid. It could not be extracted into acid solution after shaking with sodium hydroxide solution, and its absorption spectrum was markedly different from that of mepacrine. It is therefore evident that chick liver accumulates a metabolite of mepacrine in amounts comparable with the mepacrine present; results are given only for the basic material behaving as mepacrine. Acridine determinations were carried out at various times after treatment with mepacrine; the results are set out in Table II, which also shows the number of animals contributing to each result. The figures are mostly mean values of 2–6 determinations on smaller sub-groups of animals. Where marked discrepancies were found the determinations were repeated.

The figures show certain distinctive differences in the distribution of acridine in the tissues. Mouse and rat were outstanding in showing liver concentrations rising gradually to a high peak. In mouse liver the peak was about twice the 6-hour level and was reached in about 24 hours, but in rat liver the peak was five times the 6-hour level and was reached later at about 4 days after treatment. By contrast, in the other species the maximum was almost reached in six hours. In guinea-pig and chick this concentration began to fall after 24 hours, but in the rabbit the rather low maximum was maintained with

TABLE II

TOTAL ACRIDINE IN ORGANS OF DIFFERENT SPECIES AFTER A SINGLE ORAL DOSE OF MEPACRINE

For details of dose see text. Total acridine as μg. mepacrine salt/g. moist tissue. Figures in parentheses show number of animals contributing to each mean value.

Organ	Species	Content at Given Time after Treatment							
		3 hr.	6 hr.	12 hr.	24 hr.	2 days	4 days	8 days	16 days
Liver	Mouse.. ..	950 (12)	1,350 (24)	1,840 (42)	2,330 (24)	1,560 (18)	1,330 (12)		80 (12)
	Rat		520 (2)	680 (2)	1,630 (2)	2,300 (6)	2,520 (2)	2,550 (6)	650 (2)
	Rabbit.. ..		430 (4)	430 (5)	480 (2)	610 (5)	560 (2)	60 (6)	70 (5)
	Guinea-pig ..		640 (4)	710 (3)	810 (4)	640 (7)	120 (4)		
	Chick* ..		680(120)	530(120)	880 (60)	700 (30)	300 (30)		8 (30)
Spleen	Mouse.. ..	480 (12)	350 (42)	770 (24)	450 (24)	390 (18)	120 (12)		30 (12)
	Rat		160 (2)	190 (2)	330 (2)		640 (8)	410 (6)	390 (2)
	Rabbit.. ..		610 (4)	470 (5)	500 (2)	400 (5)		60 (3)	30 (2)
	Guinea-pig ..		420 (4)	310 (3)	700 (4)	610 (3)	130 (4)		
	Chick		650 (60)	1,080 (90)	1,040 (30)	1,210 (30)	390 (30)		0 (30)
Kidney	Mouse ..	420 (12)	520 (42)	690 (42)	450 (24)	370 (18)	170 (12)		50 (12)
	Rat		120 (2)	90 (2)	200 (2)	400 (6)	450 (8)	420 (6)	310 (2)
	Rabbit.. ..		570 (4)	420 (5)	390 (2)	620 (5)	740 (2)	180 (3)	110 (2)
	Guinea-pig ..		170 (4)	270 (3)	370 (4)	220 (3)	150 (4)		
	Chick ..		180 (60)	190 (90)	250 (30)	100 (30)	50 (30)		1 (30)
Brain	Mouse.. ..		16 (12)	28 (12)	16 (12)		20 (12)		12 (12)
	Rat		7 (2)	5 (2)	8 (2)		14 (2)		7 (2)

* Results for chick liver refer only to acridine extractable into acid solution.

little change for at least 4 days. The acridine concentrations in the spleen, compared with the kidney of the same species at any particular interval after treatment, were closely similar in the mouse, rat, and rabbit. In the guinea-pig and chick the spleen levels were much higher than those in the kidney, but changes in the two organs at different times after treatment were roughly parallel. For the rat and mouse the maximum spleen values were a small fraction of those in the liver, whereas in the other species the level in the spleen approached or even exceeded that in the liver. Acridine concentrations in spleen and kidney usually showed a relatively small rise after 6 hours to a maximum, followed by a slow decline. The maximum occurred in mice and chicks at 12–24 hours, in guinea-pigs at 24–48 hours, and in rats and rabbits at about 4 days. The persistence of acridines in the rat was most marked. Substantial amounts remained in liver, spleen, and kidney 16 days after treatment, whereas in all other species the values had dropped markedly. A similar observation with rats was made by Scudi and Hamlin (1943).

Considering these results, it appeared that the only characteristic which distinguished the mouse from all the other species in the distribution of acridines was the rapid development of very high concentrations in the liver. Equally high levels were eventually reached in the rat, but only after a delay of several days. If the requirement for antiviral activity were the presence of a high concentration of acridine in the liver within a few hours of virus inoculation, it might be expected that mepacrine would show a good antiviral effect in the rat, provided that infection were postponed until 3–4 days after treatment with the drug. Experiments to test this possibility were therefore carried out.

The Effect of Mepacrine in the Mouse and Rat According to the Time of Administration Relative to That of Infection

In the mouse we have consistently obtained the maximal therapeutic effect of mepacrine when the drug has been given at the time of, or 24 or 48 hours before, infection with Eastern equine encephalomyelitis. In older animals reasonable, or sometimes quite striking, effects have followed administration 4 or 24 hours after virus, but, even in these, prior treatment with mepacrine has yielded better results. This suggests that it is necessary for a period to elapse, either to enable some metabolite to be formed or for mepacrine itself to become closely associated with some critical cellular component, before extraneural infection is fully established if the maximal therapeutic response is to be obtained. If the antiviral effect were directly related to the concentration of drug in the liver, it might be expected that the temporal relations would be altered as mentioned in the previous paragraph. The data presented in Table III give no indication of this. With the very young (and more highly susceptible) mice used, the therapeutic effect was less marked than in older animals, but it is clear that the optimal period for administration of drug is as stated above, and that in this respect no difference exists between the mouse and the rat. However, the fact remains that the concentration of mepacrine in the liver of mouse and rat is out-

TABLE III

EFFECT OF TIME OF TREATMENT ON THE THERAPEUTIC RESPONSE TO MEPACRINE IN THE MOUSE AND RAT

Figures show number of deaths in groups of 20, and mean periods (days) of survival in parentheses.

Time of Treatment	Expt. 1		Expt. 2	
	Mice	Rats	Mice	Rats
No drug	20 (4·6)	5 (7·0)	20 (5·3)	10 (6·1)
5 days before virus	16 (7·0)	4 (6·8)	14 (6·1)	5 (5·2)
2 „ „ „	9 (7·6)	1 (8·0)	9 (7·8)	3 (6·6)
1 day „ „	7 (8·1)	0	6 (8·2)	1 (7·0)
1 „ after „	19 (4·2)	3 (8·7)	17 (4·5)	2 (5·5)

standingly higher than in species insusceptible to chemotherapy with the drug.

Separation of Cell Components and Determination of Mepacrine Contents

The method of separation used was essentially that of Hogeboom, Schneider, and Pallade (1948) with some modifications. The preparations were examined microscopically as fixed and stained smears on slides. The fractionation was designed to yield fairly pure preparations of nuclear, mitochondrial and microsomal particles together with intermediate fractions containing mixtures of particles. Any separation of this kind must be a compromise between high yield and high purity of any particular cell component. Consequently it is difficult to estimate the total content of a drug in any particular component. In these experiments the high mepacrine content of the mitochondrial fraction in relation to other fractions made the task somewhat simpler, since small amounts of microsomes and cell plasma contaminating the mitochondrial fraction would contribute little to the total acridine present. The results are shown in Table IV. It will be seen that in mouse liver, after a single large dose of mepacrine, the acridine tends to be concentrated mainly in the mitochondrial particles, and to a less extent in the nuclei, with relatively low concentrations in the microsomal particles and the cell plasma. The ratio of acridine present in the mitochondrial particles to that in the nuclei rises from about 2 at 3 to 6 hours after treatment to 4 at 2 to 4 days and then declines again. At the peak of acridine concentration in the mouse liver 60–70% is found in the mitochondrial particles. Figures for the cell components of the guinea-pig are less extensive, but serve to show that in this species also the amounts of acridine in the mitochondrial particles are greater than in any of the other cell components. The acridine ratio (mitochondrial component/nuclei) varies from 3 at 6 hours after treatment to less than 2 at 24 and 96 hours.

The results of the distribution of acridine in mouse liver components are in direct contrast to those of Tomkins and Brodie (1954) for rat livers. Brodie claims that mepacrine is very largely concentrated in the nuclei where it is firmly held. A single experiment, under our conditions, with rats suggested that in rats as in mice and guinea-pigs the main concentration was in the mitochondrial particles.

TABLE IV

TOTAL ACRIDINES IN CELL COMPONENTS OF MOUSE AND GUINEA-PIG LIVER AT VARYING TIMES AFTER A SINGLE DOSE OF MEPACRINE

Mean total acridine as μg. mepacrine salt/g. liver.

Separated Fractions	Animal:	Mouse								Guinea-pig		
	Time after dose (hr.):	3	6	12	24	48	96	190	380	6	24	190
	No. of separate expts.:	2	3	2	2	2	3	2	1	2	1	1
A		120	140	190	330	310	200	140	7	55	120	9
B		160	220	230	210	210	160	150	7	64	76	12
C		720	960	1,300	2,120	2,150	1,340	580	40	520	520	84
D		590	700	930	1,220	1,360	1,140	450	30	330	340	22
E		170	280	520	750	690	450	340	20	65	160	21
F		80	70	200	370	320	150	50	0	77	64	14
G		80	80	110	110	190	60	20	4	74	53	6
H		80	120	70	150	240	90	30	4	85	65	28
Calculated Content of Components												
Nuclear		280	360	420	540	520	360	290	14	120	200	21
Mitochondrial ..		560	690	1,230	1,800	1,850	1,380	540	36	360	370	36
Microsomal ..		160	160	220	220	380	120	40	8	150	110	6
Cell plasma ..		80	120	70	150	240	90	30	4	85	65	28
Total		1,080	1,330	1,940	2,710	2,990	1,950	900	60	715	745	91
Acridine ratio: mitrochondrial/ nuclear fractions		2·0	1·9	2·9	3·3	3·6	3·8	1·9	2·6	3·0	1·9	1·7

It seems likely that the distribution depends on the dose administered. Brodie's conditions (private communication) were daily intraperitoneal doses of mepacrine at 50 mg./kg. He was later able to confirm our results with large oral doses.

Discussion

Although the pattern of distribution of mepacrine in different organs varies considerably from one species to another, the only feature which is clearly characteristic of the mouse is the rapid establishment of high levels of acridine in the liver; in the rat similar levels are reached, but only after some delay. The evidence presented suggests that, although there may be a correlation between the high mepacrine levels in the livers of these two species and the antiviral action of the drug, there are certainly other unknown factors involved.

The distribution of drugs within the cell itself has rarely been studied. The present investigation has provided an example of the possibility of doing so, and of some of the precautions required in interpreting the results. It has also shown that mepacrine can be largely concentrated in a particular cell component—under the conditions of these experiments in the mitochondrial fraction—and that the relative concentrations in different parts of the cell may change at different times after treatment.

The possible correlation between the local concentration of mepacrine within the liver cell and its antiviral effect can only be considered in relation to the distribution of virus within the cell. This has been the subject of further experiments which will be discussed in a later publication.

Summary

1. Concentrations of mepacrine in liver, spleen, and kidney resulting from a single massive dose have been measured in mice, rats, guinea-pigs, chicks, and rabbits at different times after administration.

2. Mice and rats—the only species in which mepacrine exhibits a protective action against infection with Eastern equine encephalomyelitis—are distinguished by development of exceptionally high concentrations in the liver. Although this correlation may be significant, evidence suggests that other factors must play a part in determining the antiviral action of mepacrine.

3. With the dose of mepacrine used, the bulk of the acridine in the mouse liver was in the mitochondrial fraction, with decreasing amounts in the nuclear, microsomal and cell plasm fractions respectively. The difference in concentrations between the components was greatest when the total acridine in the organ was at a maximum. Distribution of the drug in the components of guinea-pig liver was probably similar.

The authors wish to thank Messrs. J. H. Sherwood and J. C. B. Smith for technical assistance in analysis.

References

Brodie, B. B., and Udenfriend, S. (1943). *J. biol. Chem.*, **151**, 299.

Dearborn, E. H., Kelsey, F. E., Oldham, F. K., and Geiling, E. M. K. (1943). *J. Pharmacol.*, **78**, 120.

—— (1947). Ibid., **91**, 174.

Goodall, R. R. (1956). *Brit. J. Pharmacol.*, **11**, 215.

Hecht, G. (1936). *Arch. exp. Path. Pharmak.*, **183**, 87.

Hogeboom, G. H., Schneider, W. C., and Pallade, G. E. (1948). *J. biol. Chem.*, **172**, 619.

Hurst, E. W., Melvin, P., and Peters, J. M. (1952). *Brit. J. Pharmacol.*, **7**, 455.

—— Peters, J. M., and Melvin, P. (1952). Ibid., **7**, 473.

—— Snow, G. A., and Roberts, D. C. (1955). *Brit. J. exp. Path.*, **36**, 215.

Oldham, F. K., and Kelsey, F. E. (1945). *J. Pharmacol.*, **83**, 288.

Scudi, J. V., and Hamlin, M. T. (1943). *Proc. Soc. exp. Biol., N.Y.*, **54**, 127.

Tomkins, G., and Brodie, B. B. (1954). *Fed. Proc.*, **13**, 411.