Brit. J. Pharmacol. (1948), **3**, 198.

CIRCULATORY PROPERTIES OF AMIDINE DERIVATIVES I. PRESSOR ANALOGUES OF METHYL *ISO*THIOUREA

BY

F. N. FASTIER

From the Department of Medicine, Otago University, New Zealand

(Received March 6, 1948)

The experiments to be described in this and the succeeding paper are concerned primarily with the circulatory properties of methyl *iso*thiourea in relation to its physico-chemical constitution.

Two considerations prompted such a study: First, the chemical structure of methyl *iso*thiourea is not at all complex; a diversity of related compounds were readily accessible. Secondly, the pharmacological properties by which methyl *iso*thiourea was known to be distinguished (Smirk, 1941; McGeorge, Sherif, and Smirk, 1942) seemed likely to permit the rapid testing of chemically related substances. A pressor action is the most noteworthy of these properties, and, as the substance has been employed therapeutically for maintaining the blood pressure in spinal anaesthesia (Smirk and McGeorge, 1942), the possibility of finding other useful pressor agents was naturally a further inducement to the study of related substances.

Screening experiments involving a wide range of compounds have already received brief comment (Fastier, 1944). Some of the points then raised are dealt with more fully in the present report, whose main purpose it is to outline difficulties in the way of a satisfactory explanation of the distribution of pressor activity among substances chemically related to methyl *iso*thiourea.

METHODS

Commercial samples of iminoazole (Kodak) and 2-aminopyridine (L. Light) were used. S,N-ethylene *iso*thiourea hydrobromide was prepared from β-bromoethylamine hydrobromide and potassium thiocyanate, and S,N-propylene *iso*thiourea hydrochloride by the action of warm concentrated hydrochloric acid on allyl thiourea. The effects of these N-substituted amidine derivatives upon the blood pressure of anaesthetized dogs and cats, the perfusion pressure of pithed rat hind-quarters, and the tonus of isolated strips of rabbit intestine were recorded by methods used in earlier studies (Fastier and Smirk, 1943, 1947).

Methyl *iso*thiourea methylsulphate and methylene di-*iso*thiourea dihydriodide were synthesized by alkylating thiourea with dimethyl sulphate and methylene iodide respectively. Their higher homologues were obtained as hydrobromides by the method of Sprague and Johnson (1937). When no reference to the melting point of the recrystallized salt was found in the literature its composition was checked by a halide determination. Attention was restricted (so far as this paper is concerned) to the blood pressure responses of anaesthetized animals to these other amidine derivatives, except with hexamethylene di-*iso*thiourea; the cardiac effects of the latter were studied (i) in anaesthetized cats by slipping a small glass cardiometer over the heart after the thorax had been opened medially and artificial respiration applied, (ii) in rabbits by perfusing the isolated heart with Ringer-Locke solution by a technique described previously (Fastier and Smirk, 1943), and (iii) in anaesthetized dogs by inserting a long glass cannula into the left jugular vein almost down to the auricle. Clotting was prevented in the last type of experiment by filling the system connecting the cannula to a water manometer with a 5 per cent (w/v) solution of chlorazol fast pink, a little of which was run into the circulation every few minutes. The action of hexamethylene di-*iso*thiourea on the arterial blood pressure of decerebrated and pithed cats, and of anaesthetized and unanaesthetized grey rabbits (method of Grant and Rothschild, 1934), was also investigated, as were its effects on perfused rat hind-quarters and excised rabbit gut.

RESULTS

Influence of chain-length upon pressor activity

One conclusion reached on the basis of the above-mentioned screening experiments is that the pressor activity of basic amidine derivatives is affected adversely by an increase in the length of

side-chains. To discover to what extent "ionic complexity" plays a part in determining activity, twelve homologues of methyl *iso*thiourea of general formula* $CH_3(CH_2)_nS.C(:NH_2^+)NH_2$ have been synthesized and their circulatory effects compared. These alkyl *iso*thioureas provide a series in which a gradual but ultimately considerable variation in the physical properties of the kation is obtained with minimal deviation from the chemical structure of the prototype, methyl *iso*thiourea (for which $n=0$).

*Represented conveniently as kations for reasons given before (Fastier and Smirk, 1947).

The results illustrated in Fig. 1 need little explanation. It will be seen that lengthening the alkyl side-chain produces a notable alteration in the blood pressure response to a moderate initial dose (1–10 mg./kg.) of the *iso*thiourea salt. As n

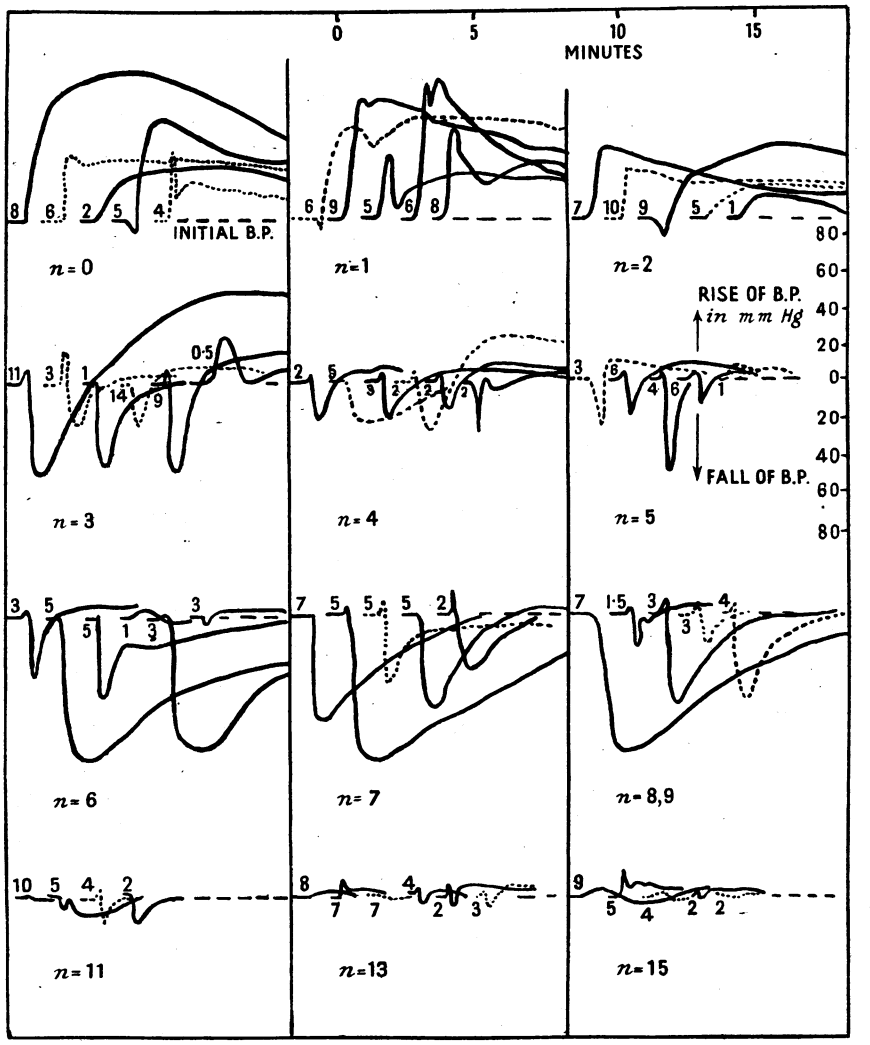


FIG. 1.—Effects on the blood pressure of anaesthetized dogs of *iso*thiourea derivatives of general formula $CH_3(CH_2)_nS.C(:NH_2^+)NH_2$. The curves are facsimiles of kymograph tracings recorded after the intravenous injection of a salt of one of the *iso*thioureas; n refers to the particular one used. The dose in mg./kg. is given alongside each curve. Dotted curves indicate experiments where vagotomy was not performed.

increases, the ability to produce a large, well-maintained rise of blood pressure is soon lost. In contrast to methyl and ethyl *iso*thiourea, the hexyl, heptyl, octyl, and nonyl homologues are predominantly depressor, when given under the same conditions, but the last three tested ($n=11$, 13, 15) are almost inert.

It should be added that the phenomenon of tachyphylaxis, already observed with various other amidine derivatives (Fastier and Smirk, 1943, 1947), was noticed with most of these *iso*thiourea salts. Since the response to successive equal doses of a given *iso*thiourea may change considerably in the course of an experiment and since treatment with one *iso*thiourea often modifies at the same time the response to a related compound no less markedly, it is difficult to make a satisfactory comparison of the effects of several *iso*thiourea salts in

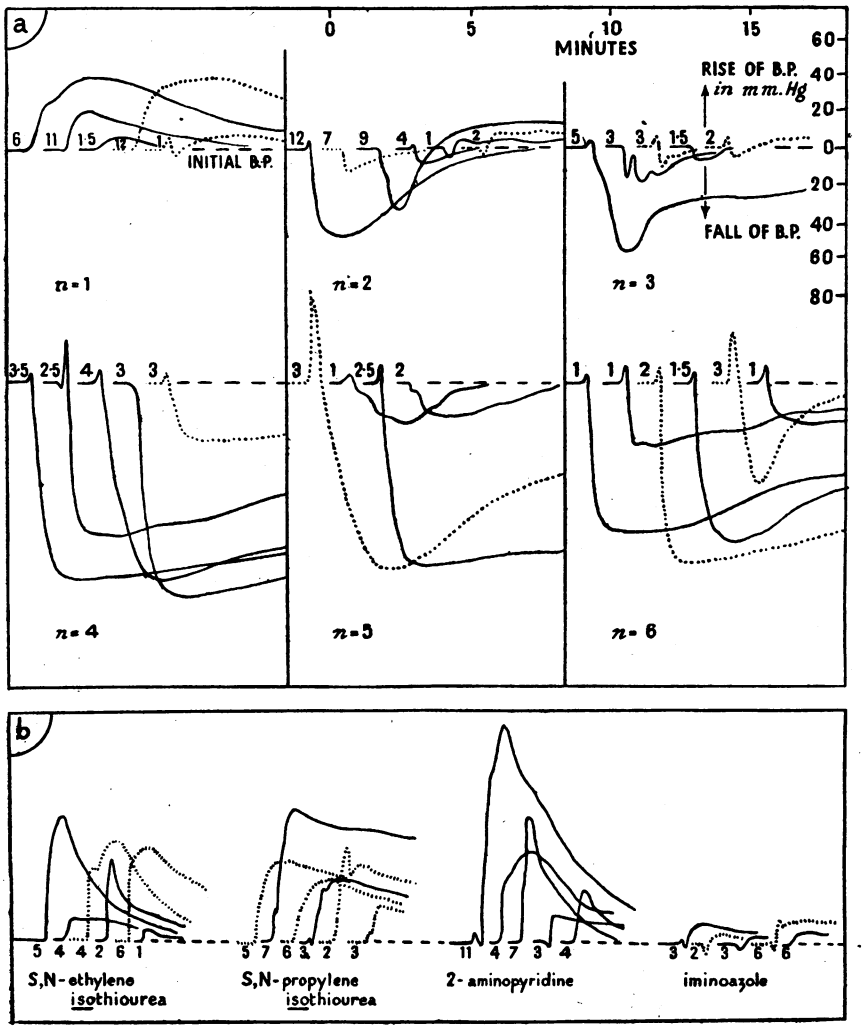


FIG. 2.—Effects on the arterial blood pressure of anaesthetized dogs and cats of (a) di-*iso*thioureas of general formula $H_2N(^+H_2N:)C.S(CH_2)_nS.C(:NH_2^+)NH_2$, and (b) certain N-substituted amidine derivatives. As in Fig. 1, the curves are facsimiles of those recorded after the initial injection of the compound. The dose in mg./kg. is appended to each curve. Experiments on cats are indicated by dotted curves. In (a), *n* refers to the particular di-*iso*thourea used.

the one animal. For this reason, more reliance has been placed upon a comparison of the effects of initial doses of each *iso*thiourea in fresh preparations.

Depressor action of iso*thiourea derivatives, as exemplified by hexamethylene di-*iso*thiourea dihydrobromide*

Depressor activity was found to be even more striking in the higher members of the di-*iso*thiourea series of general formula:

$$H_2N(^+H_2N:)C.S(CH_2)_nS.C(:NH_2^+)NH_2.$$

Only methylene di-*iso*thiourea ($n=1$) possesses pressor activity at all comparable with that of methyl *iso*thiourea (Fig. 2). The interest of the higher members tested ($n=2$–6) lies rather in the sustained falls of blood pressure they can elicit even when injected in fairly small amounts (1–5 mg./kg.). Further experiments were therefore performed

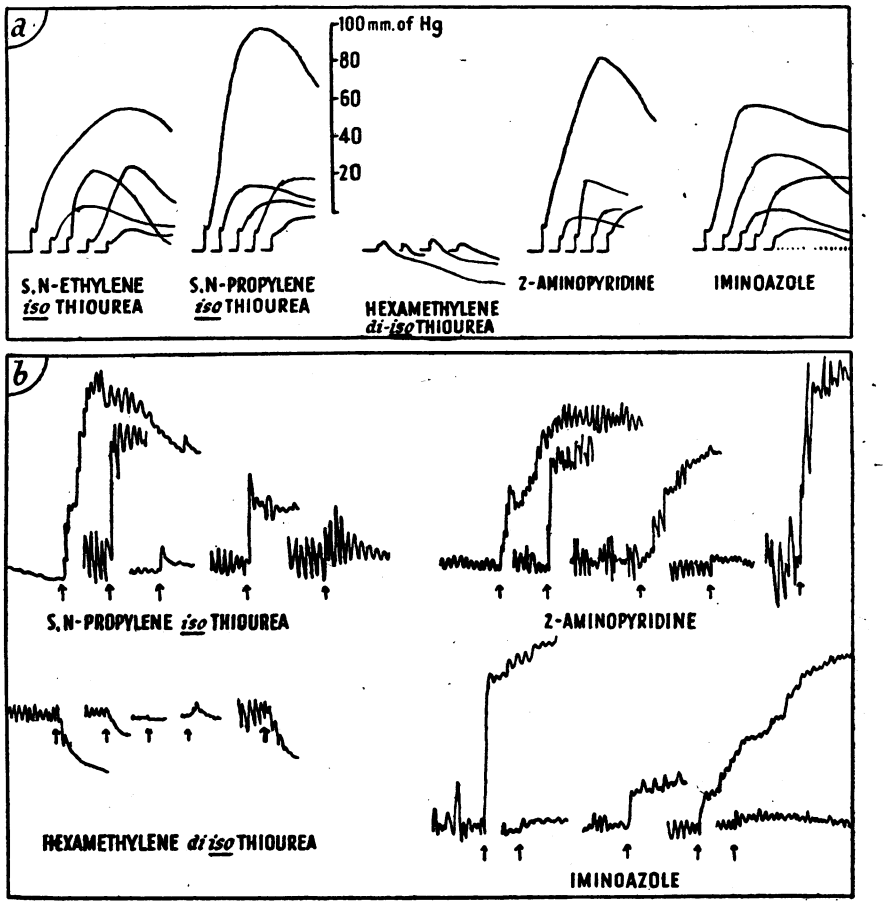


FIG. 3.—Facsimiles of kymograph records showing effects of certain amidine derivatives on (a) pithed rat hind-quarters perfused with Ringer-Locke solution containing sufficient ergotoxine (1: 200,000) to "reverse" the normal vasoconstrictor effect of adrenaline, and (b) excised rabbit intestine rendered insensitive to acetylcholine by atropinization.

The N-substituted amidine derivatives tested constrict the perfused blood vessels and cause the contraction of the gut in the dose given (0.1 c.c. of the *M*/10 solution) even in the presence of these blocking agents. The "higher" amidine derivative hexamethylene di-*iso*thiourea shows little resemblance to them in either preparation when injected in a corresponding dose (0.1 c.c. of the *M*/20 solution).

with the hexamethylene derivative in order to discover how the fall of blood pressure comes about.

In all of 15 animals anaesthetized with sodium barbitone—7 dogs, 6 cats, and 2 rabbits—the intravenous injection of hexamethylene di-*iso*thiourea dihydrobromide caused a persistent fall of blood pressure. The falls obtained in dogs with doses of 0.5–2.0 mg./kg. ranged from 25–110 mm. Hg and lasted for from fifteen to upwards of sixty minutes (Fig. 2). Definite falls of blood pressure were produced in unanaesthetized as well as in anaesthetized grey rabbits.

Effects on cardiac output, so far as they could be discerned, did not suggest that the fall of blood pressure was due to cardiac depression. The *iso*thiourea did not reduce significantly the strength of contraction of isolated rabbit hearts when perfused in concentrations of up to 1 in 25,000; nor was there evidence of depression after its injection in 4 experiments on cats in which cardiometer records were obtained while the blood pressure was falling; rather the reverse. In dogs the venous pressure was lowered by some 10–30 mm. H_2O in 2 out of 4 experiments after a temporary rise. Electrocardiograms taken at the time did not reveal any noteworthy changes in rhythm. The heart rate was usually found to be slowed when noted five to ten minutes after the injection of hexamethylene di-*iso*thiourea, sometimes after an initial acceleration. Vagotomy, which was performed in 7 out of 13 experiments, did not affect the fall of blood pressure.

When a dilator action on blood vessels is thus indicated, it is difficult to prove that the action is a direct one. Hexamethylene di-*iso*thiourea was found to lower the blood pressure in decerebrated cats, but it did not have this effect when the animal was pithed, so long as the blood pressure remained at the low level to which it had fallen subsequent to the latter operation. A vasodilator action was inconspicuous also in the pithed rat hind-quarters preparation except when the pressure had been raised beforehand by treatment with adrenaline or propionamidine. The possibility therefore remains that its vasodilator action in the latter circumstance results from interaction with the vasoconstrictor drug, and not merely from the restoration of some degree of tonus to the blood vessels. It was noted in this connexion that much smaller vasodilator responses to hexamethylene di-*iso*thiourea were obtained when adrenalytic concentrations of ergotoxine were used in place of adrenaline or propionamidine to constrict the perfused rat blood vessels (Fig. 3). A direct inhibitory action on smooth muscle was more definitely suggested by its effect on excised rabbit intestine; in a bath concentration of $M/100{,}000$ or upwards, hexamethylene di-*iso*thiourea was found to depress the tonus and spontaneous movements of the strip (Fig. 3), and to make it at the same time much less sensitive to the action of acetylcholine.

Pressor effects of some N-substituted amidine derivatives

It had been noted on the basis of screening experiments (Fastier, 1944) that pressor activity is distributed fairly widely among amidine derivatives. Inasmuch as the structural relationship to methyl *iso*thiourea (I) of pressor analogues like 2-aminopyridine (III) and iminoazole (IV) is far from close, the possibility must be considered, especially in view of the above results, that their pharmacological resemblance to it is merely superficial. The pressor actions of these particular amidine derivatives have therefore been analysed in some detail, along with those of S,N-ethylene *iso*thiourea (II) and S,N-propylene *iso*thiourea, N-substituted amidine derivatives whose structural relationship to methyl *iso*thiourea is more apparent.

(I) $CH_3.S-C(=NH_2^+)-NH_2$

(III) $CH=CH-C(-CH=CH-NH^+)=NH_2$

(II) $CH_2.S-C(=NH^+)-NH_2$ with CH_2 bridging to the NH^+

(IV) $H-C(=NH^+-CH)-NH-CH$

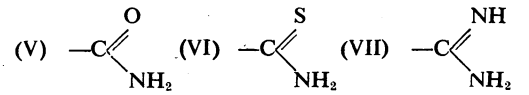
The rises of blood pressure produced in anaesthetized animals by the above bases in doses of the order of 1–10 mg./kg., though less persistent as a rule than those produced by methyl *iso*thiourea under similar conditions, were fairly striking except in the case of iminoazole (Fig. 2b). All four constricted perfused rat blood vessels about as strongly as methyl *iso*thiourea (Fig. 3a), their tonus-increasing effects on this preparation and on isolated rabbit intestine standing in sharp contrast to those of hexamethylene di-*iso*thiourea. Vasoconstrictor effects were not appreciably antagonized by treatment with ergotoxine. Moreover, atropine was usually ineffective in antagonizing their excitatory effects on gut (Fig. 3b).

Discussion

So long as we remain ignorant of the exact site of action of methyl *iso*thiourea we cannot say with certainty that *any* of its chemical relatives act in essentially the same manner, however likely this

may appear when various pharmacological effects are compared. It is tempting to assume that the pharmacological similarity to methyl *iso*thiourea of its various pressor analogues has a chemical basis. Nevertheless such an assumption is reasonable only in so far as it can be shown that these pressor analogues have in common with methyl *iso*thiourea either structural or physical features which are not shared with inactive relatives.

The strong basicity of methyl *iso*thiourea has already been stressed in this connexion (Fastier, 1944). Pressor activity has been found to be inconspicuous, if present at all, in imino-ethers and such other comparatively weakly basic chemical relatives as the ureas, carbamates, thiocarbamates, thioureas, and thiohydantoins which have the amide (V) or thioamide (VI) but not the structurally similar amidine (VII) group. Even amongst the amidine derivatives used in these preliminary experiments, striking pressor activity was observed only with those that ionize freely.



But while possession of a strongly basic character therefore seems to be a necessary condition for pressor activity like that exhibited by methyl *iso*thiourea, it has not proved possible to correlate what appears to be a fairly specific pharmacological action with possession of anything very specific from a structural viewpoint in the way of a " pharmacophoric " or " key " group. In previous reports (Fastier and Smirk, 1943, 1947), it has been shown that pressor activity of apparently the same origin is exhibited not only by homologues like ethyl and *iso*propyl *iso*thiourea but also by various other amidine derivatives of general formula $X.C(:NH_2{+})NH_2$—e.g., methyl *iso*-urea, ethyl *iso*-urea, propionamidine, methylguanidine, and *asym*-dimethylguanidine, where $X = CH_3O$-, C_2H_5O-, C_2H_5-, CH_3NH-, and $(CH_3)_2N$- respectively. Salts of all these bases have been shown to cause, amongst other effects, constriction of perfused blood vessels, even in the presence of strongly adrenalytic concentrations of ergotoxine, and contraction of atropinized gut: evidence which suggests that they owe their pressor activity in part at least to a capacity to constrict blood vessels by a direct action on their musculature. In so far as 2-aminopyridine (III), iminoazole (IV), and other N-substituted amidine derivatives referred to above satisfy the same criteria, their pharmacological resemblance to methyl *iso*thiourea is equally convincing.

It will be noticed that these various musculotropic bases have nothing more in common from a structural viewpoint than the amidine group -C(:NH-+)NH-. How then are we to account for the finding (Fig. 1) that merely lengthening the alkyl side-chain of methyl *iso*thiourea seems to bring about a reversal of activity ?

Presumably, if these pressor analogues of methyl *iso*thiourea do have a fundamentally similar mode of action, we must give chief consideration to " physical " as distinct from purely structural attributes in trying to explain their pharmacological similarity on a chemical basis. The impression that their pressor activity is generally affected adversely by increasing the length of side-chains may therefore be highly significant, and this clue will be followed up in succeeding papers.

Summary

1. In the series of *iso*thioureas of general formula $CH_3(CH_2)_nS.C(:NH)NH_2$, pressor activity is affected adversely by increasing the length of the side-chain (for $n=0$–9). Only the first three members are able to produce large, persistent rises of blood pressure in anaesthetized dogs.

2. Likewise in the di-*iso*thiourea series of general formula $H_2N(HN:)C.S(CH_2)_nS.C(:NH)NH_2$, depressor effects rapidly become predominant as the series is ascended ($n=0$–6). The falls of blood pressure caused by the hexamethylene derivative when given in doses of 2–5 mg./kg. are particularly large and long-lasting; they are brought about mainly, if not entirely, through vasodilatation.

3. Although their structural relationship to methyl *iso*thiourea is much less obvious than that of the above *iso*thioureas, the N-substituted amidine derivatives 2-aminopyridine and iminoazole resemble it closely in their circulatory effects.

4. Like S,N-ethylene and S,N-propylene *iso*thiourea—and also various other amidine derivatives of low molecular weight examined previously (Fastier and Smirk, 1943, 1947)—2-aminopyridine and iminoazole constrict perfused rat blood vessels even in the presence of strongly adrenalytic concentrations of ergotoxine, and cause the contraction of atropinized gut; thus they appear to act directly on the smooth muscle of blood vessels.

5. It is concluded that if the pharmacological resemblance to methyl *iso*thiourea of these various

pressor analogues has indeed a chemical basis, " physical " as distinct from purely structural attributes must play a large part in determining pressor activity in compounds of this type.

This work has been aided by a grant from the New Zealand Medical Research Council. I wish to acknowledge also my indebtedness to Prof. F. H. Smirk for much helpful criticism.

References

Fastier, F. N. (1944). *Nature, Lond.*, **154**, 429.
Fastier, F. N., and Smirk, F. H. (1943). *J. Physiol.*, **101**, 379.
Fastier, F. N., and Smirk, F. H. (1947). *J. Pharmacol.*, **89**, 256.
Grant, R. T., and Rothschild, P. (1934). *J. Physiol.*, **81**, 265.
McGeorge, M., Sherif, M., and Smirk, F. H. (1942). *J. Physiol.*, **100**, 474.
Smirk, F. H. (1941). *Brit. med. J.*, **2**, 510.
Smirk, F. H., and McGeorge, M. (1942). *Lancet*, **2**, 301.
Sprague, J. M., and Johnson, T. B. (1937). *J. Amer. chem. Soc.*, **59**, 1837.