has been interpreted as suggesting a possible relation to the mechanism of antiinflammation; however, these results suggest that any such relation is not specific.

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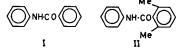
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References

Sancilio, L. F. & Rodriguez, R. (1965). Fedn Proc. Fedn Am. Socs exp. Biol., 24, 677.
Skidmore, I. F. & Whitehouse, M. W. (1965). J. Pharm. Pharmac., 17, 671–673.
Whitehouse, M. W. & Skidmore, I. F. (1965). Ibid., 17, 668–670.
Winter, C. A., Risley, E. A. & Nuss, G. W. (1962). Proc. Soc. exp. Biol. Med., 111, 544–547.

The influence of the stability of the amide link on the formation of methaemoglobin by anilides

SIR,—It is considered that the reactions involved in the formation of methaemoglobin by aromatic amides are (a) hydrolysis of the amide, (b) metabolism of the amine produced to the appropriate species, and (c) oxidation of the haemoglobin by the amine metabolite (McLean, Murphy & others, 1967). The stability of the amide link in anilides may be modified by (a) substitution on the aromatic ring, and (b) substitution on the acyl group. McLean & others (1967) examined a wide range of derivatives of acetanilide which were substituted on both the acyl and aromatic moieties and found no correlation between the stability of the amide group and the ability of the compounds to induce the formation of methaemoglobin in cats. They came to the conclusion that the nature of the amine formed by hydrolysis of the aromatic amide was of prime importance in determining the amount of methaemoglobin formed by the compounds. We wish to report an example in which the hydrolysis of an anilide is the rate determining step in the formation of methaemoglobin. It is well known that disubstitution in the 2,6-positions of an aromatic amide or ester confers considerable stability on the amide or ester group because of the socalled "ortho-effect". McLean & others (1967) attempted to determine the importance of the "ortho-effect" in controlling the ability of an aromatic amide to form methaemoglobin by examining a series of 2,6-dimethylanilides. These amides formed much less methaemoglobin than the corresponding unsubstituted anilides but the influence of the "ortho-effect" could not be assessed because 2.6-dimethylaniline was also a poor former of methaemoglobin. To obtain evidence that the rate of hydrolysis of an anilide can be the rate determining step in the formation of methaemoglobin, benzanilide (I) and 2', 6'-dimethyl-



benzanilide (II) have now been examined. The compounds were prepared by condensing the appropriate acid chloride with aniline. 2,6-Dimethylbenzoic acid was prepared by the method described by Thomas & Canty (1962) and converted to 2,6-dimethylbenzoyl chloride by treatment with thionyl chloride. Benzanilide was recrystallized from benzene-ethanol as white crystals, m.p.

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163–164° (found: C, 78·8; H, 5·6; N, 7·0. $C_{13}H_{11}NO$ requires C, 79·1; H, 5·6; N, 7·1%). 2′,6′-Dimethylbenzanilide was recrystallized from ethyl acetateligroin as white needles m.p. 138·5° (found: C, 79·9; H, 6·4; N, 6·2. $C_{15}H_{15}NO$ requires C, 80·0; H, 6·7; N, 6·2%). The ability of the compounds to form methaemoglobin in cats was determined by the method described by McLean & others (1967). The results obtained, together with the results which had previously been obtained for acetanilide and aniline are given in Table 1.

Compound		Dose	No. of cats	Mean % Met-Hb formed Time after admin. of compound (hr)						Mean % Met-Hb
				1	2	3	4	5	6	formed over total time
Acetanilide		0.5 mmole/kg	5	38.0	66-9	73.5	78.1	76.6	78.5	68.6
Benzanilide	••	1 mmole/kg oral	5	17.8	41.4	43.1	33.4	27.1	20.5	30.6
2',6'-Dimethyl- benzanilide		1 mmole/kg oral	2	1.8	1.2	1.2	0.5	2.3	0.7	1.1
Aniline		0.25 mmole/kg i.v.	5	70.6	70.0	66.6	64.4	55.6	-	65.4

 TABLE 1.
 Methaemoglobin (% total haem pigments) formed in cats after administration of aniline and some anilides

It can be seen that aniline (0.25 mmole/kg i.v.) and acetanilide (0.5 mmole/kg, oral) formed approximately the same amount of methaemoglobin, while benzanilide (1 mmole/kg, oral) formed about half as much, and 2',6'-dimethylbenzanilide (1 mmole/kg, oral) formed virtually none. Since with all these compounds the methaemoglobin produced is related ultimately to the concentration of aniline present in body fluids, the conclusion to be drawn is that the slow rate of hydrolysis of 2',6'-dimethylbenzanilide *in vivo*, and to a lesser extent of benzanilide, is the overall rate determining step in the formation of methaemoglobin by these compounds. The conclusion from these results considered along with the results of McLean & others (1967) is that it is possible to modify the ability of an aromatic amide to form methaemoglobin by retarding hydrolysis of the amide group but this is only manifest when profound changes are made in the reactivity of the amide link.

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References

 McLean, S., Murphy, B. P., Starmer, G. A. & Thomas, J. (1967). J. Pharm. Pharmac., 19, 146–154.
 Thomas, J. & Canty, J. (1962). J. Pharm. Pharmac., 14, 587–596.

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