Per.OOH, but as a compound in which the iron has an effective oxidation number of +4.

2. These results strongly support the following new mechanism for the interrelationship of compound I and compound II which necessitates a complete revision of the accepted mechanism for peroxidase action.

3. Preliminary values of 1.3–1.6 and approx. 1.0 V. have been estimated for the oxidation-reduc-

tion potentials of the compound I/compound II and compound II/peroxidase couples respectively at pH 5.3 (European convention).

4. Four types of possible chemical structure for compound II containing iron with an effective oxidation number of +4 are discussed, together with two general structures available for compound I, either as a ferric complex or a derivative containing iron with an effective oxidation number of +5.

I wish to thank Miss M. Grenholm, Dr A. C. Maehly and Mr T. Devlin for enzyme samples, to express my appreciation of the stimulating interest Prof. Britton Chance has shown in this work, and to acknowledge my gratitude to the Rockefeller Foundation for a Fellowship which made it possible to carry out this investigation in the Eldridge Reeves Johnson Foundation for Medical Physics, Philadelphia, from June to December 1951.

REFERENCES

- Abrams, R., Altschul, A. M. & Hogness, T. R. (1940). *J. biol. Chem.* **142**, 303.
 Altschul, A. M., Abrams, R. & Hogness, T. R. (1942). *J. biol. Chem.* **136**, 777.
 Cahill, A. E. & Taube, H. (1951). *J. Amer. chem. Soc.* **73**, 2847.
 Chance, B. (1943). *J. biol. Chem.* **151**, 553.
 Chance, B. (1949a). *Science*, **109**, 204.
 Chance, B. (1949b). *Arch. Biochem.* **22**, 224.
 Chance, B. (1949c). *Arch. Biochem.* **21**, 416.
 Chance, B. (1949d). *Arch. Biochem.* **24**, 389.
 Chance, B. (1950). *Fed. Proc. Amer. chem. Soc.* **9**, no. 1.
 Chance, B. (1951a). *Enzymes and Enzyme Systems*, ed. by Edsall, J. T. Harvard University Press.
 Chance, B. (1951b). In *Advanc. Enzymol.* **12**, ed. by F. F. Nord. New York: Interscience.
 Chance, B. (1951c). *The Enzymes*, ed. by J. B. Sumner and K. Myrbäck. New York: Academic Press Inc.
 Chance, B. (1952). *Arch. Biochem.* **37**, 235.
 George, P. (1952a). *Advanc. Catalys.* **4**, 367.
 George, P. (1952b). *Nature, Lond.*, **169**, 612.
 George, P. (1953). *Biochem. J.* (in the Press).
 George, P. & Irvine, D. H. (1951). *Nature, Lond.*, **168**, 164.
 George, P. & Irvine, D. H. (1952). *Biochem. J.* **52**, 511.
 Hume, D. N. & Kolthoff, I. M. (1943). *J. Amer. chem. Soc.* **65**, 1895.
 Keilin, D. & Hartree, E. F. (1945). *Biochem. J.* **41**, 500.
 Keilin, D. & Mann, T. (1937). *Proc. Roy. Soc. B*, **122**, 119.
 Theorell, H. (1941). *Enzymologia*, **10**, 250.
 Theorell, H. (1942). *Ark. Kemi Min. Geol.* **16A**, no. 2.
 Theorell, H., Ehrenburg, A. & Chance, B. (1952). *Arch. Biochem.* **37**, 237.
 Theorell, H., Ehrenburg, A. & Chance, B. (1953). *Arch. Biochem.* (in the Press).
 Theorell, H. & Maehly, A. C. (1950). *Acta. chem. scand.* **4**, 422.
 Walden, G. H., Hammett, L. P. & Chapman, R. P. (1933). *J. Amer. chem. Soc.* **55**, 2649.
 Woo, S. C. (1931). *J. Amer. chem. Soc.* **53**, 469.

Comparative Detoxication

1. THE METABOLISM OF SULPHADIMIDINE IN THE LOCUST

By CELIA M. MYERS AND J. N. SMITH

Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2

(Received 28 November 1952)

Knowledge of the 'detoxication mechanisms' of higher animals is finding useful application in the design of more effective drugs (cf. Brodie, 1952; Williams, 1951), and it is possible that similar studies in insects may lead to more effective insecticides (cf. Brown, 1951). Very little is known, however, of the metabolic changes undergone by foreign organic compounds in the insect. Most of the infor-

mation available deals with the dehydrohalogenation of halogenated compounds such as DDT (1:1:1-trichloro-2:2-di(*p*-chlorophenyl)ethane) (Perry & Hoskins, 1950; Ferguson & Kearns, 1949; Winteringham, Loveday & Harrison, 1951), but apart from this there are only a few isolated reports of metabolic changes in insects, for example, oxidation of salicin to salicylaldehyde and salicylic acid in

certain beetles (Hollande, 1909), the oxidation and probable conjugation of phenothiazine in the cockroach (Zukel, 1944) and the hydrolysis of pyrethrins in caterpillars (Swingle, 1934). No study has yet been made in insects of the conjugation mechanisms or oxidations which occur in animals (Williams, 1947); it is hoped to compare the known mechanisms of warm-blooded animals with those of the locust which is a large and easily obtained insect.

The conjugation reaction of the amino group was chosen for study since it can be easily followed by the Bratton-Marshall (1939) colorimetric method for aromatic amines. Furthermore, we wished to try different methods of administering compounds to locusts (cf. Shephard, 1939) and to find out how long it took the insect to excrete the drug. Sulphadimidine (Sulphamezathine, sulphadimethylpyrimidine, 2-(*p*-aminobenzenesulphonamido)-4,6-dimethylpyrimidine) was selected for the present study because, in the higher animals, it is readily absorbed from the gut, highly conjugated by acetylation and rapidly excreted (Krebs, Sykes & Bartley, 1947; Smith & Williams, 1948).

Quantitative measurements of free and total sulphadimidine have been made on excreta, and conjugated sulphadimidine calculated from the differences between these. It has not been possible to identify *N*⁴-acetylsulphadimidine in the excreta by classical methods on account of the very small quantities available; however, identity in chromatographic behaviour in three solvent mixtures between one of the excretion products and the *N*-acetyl compound is strong evidence of identity.

EXPERIMENTAL

Insects. *Locusta migratoria migratorioides* (1–2 g.) were obtained from the Anti-Locust Research Centre, London, and were kept till required in glass-fronted wooden boxes with false zinc-gauze floors of the type recommended by the Anti-Locust Research Centre (see also Chauvin, 1941). They were maintained at 30–53° and relative humidity 60–70%.

The stock locusts were fed *ad lib.* on freshly cut grass, and on this diet immature adults produced daily about 0.5 g. of faecal pellets consisting mainly of undigested food residues. These were encrusted with the solid malpighian excretions (see Uvarov, 1948), which could only be separated partially with difficulty from the bulky vegetable material.

Administration of compounds. The locusts would not feed readily on green vegetables, but after a few days deprivation they would eat small amounts of moistened biscuit ('cream crackers') or rice paper, and in some qualitative feeding experiments it was convenient to spread the compound to be fed on rice paper or to incorporate it (1–5%) into a wet mash of broken biscuit. On these diets the droppings of hoppers and immature adults were very few and appeared to consist almost entirely of the gritty pink, malpighian excretions and could be worked up free from the large amounts of pigment and carbohydrate associated with the grass diet.

In quantitative experiments, compounds were administered in solution by injection into the thorax from an Agla micrometer syringe fitted with a no. 16 needle. Alternatively, a curved blunt needle could be used and the solution injected directly into the crop. The locusts, in general, tolerated injections of acid solutions, pH 3–7, well, but alkaline solutions, pH 8, were fatal. For feeding into the crop, the syringe was clamped at 45° to the horizontal with the needle pointing upwards and the locust held in the hand; with a little practice the needle could be inserted easily through the mouth into the crop without damaging the insect. When handled, locusts usually expelled a drop of crop fluid. This was removed with filter paper from the mouth parts and then 0.01 ml. of liquid could be introduced from the syringe and was retained by the locust.

In some quantitative experiments the sulphadimidine was dissolved in acetone and a measured amount of the solution placed on to a blade of grass on which the locust was allowed to feed. When enough had been consumed the insect was removed and the sulphadimidine remaining on uneaten material estimated colorimetrically. The amount eaten could then be obtained by difference. In most experiments, however, it was found more convenient to feed the locusts with the blunt needle.

The micrometer scale of the Agla syringe was not used as a direct measure, but volumes equal to those injected were taken during each experiment and estimated colorimetrically to find the exact amount administered.

After administering the drug, the locusts were confined, either singly or in groups, in 500 ml. beakers closed with paper covers and kept in a cage at 35–40° and 60–70% r.h. The droppings were collected periodically and the locusts transferred to fresh beakers. They were fed daily on a limited amount of grass (0.1–0.2 g./locust). Some grass was necessary to form the faeces as the locusts when starved did not completely empty their intestines. On the other hand, the amount of grass fed had to be as small as possible in order to reduce the interfering brown colours of the blanks in the diazo reaction.

Quantitative estimation of conjugated sulphadimidine. The difference between the 'free' and 'total' sulphadimidine excreted represented conjugated sulphadimidine including acetylsulphadimidine. 'Free' sulphadimidine was estimated by the method of Bratton & Marshall (1939). The faecal pellets were stirred in an Atomix blender with a suitable known volume of 0.1 *N*-NaOH, filtered under pressure from any grass residues and samples of the filtrate taken for analysis. Blanks were made up from corresponding volumes of the filtrate treated in the same way, except that no naphthylethylenediamine was added. 'Total' sulphadimidine was estimated after acid hydrolysis as previously described (Smith & Williams, 1948) using a similarly treated sample of the faecal extract, without naphthylethylenediamine, as a blank. Recoveries of sulphadimidine and acetylsulphadimidine added to normal locust excreta were of the order of 90–100%.

Results with adults and hoppers are summarized in Tables 1 and 2.

Chromatographic detection of acetylsulphadimidine. Six locusts were dosed orally by syringe with 1.5 mg. each of sulphadimidine in 0.1 ml. of 2 *N*-HCl and allowed to feed on wet biscuit. The excreta, collected at 24 hr. and 48 hr., were ground in a mortar and extracted twice with 2.0 ml. of 4:1 (v/v) acetone/water mixture. After filtration and

Table 1. *Free and total sulphadimidine in locust excreta*

Stage	Amount eaten ($\mu\text{g.}$)	1st day			2nd day			3rd-5th days*			Total in exuvia ($\mu\text{g.}$)	Recovery (%)	Conjugates (as % of recovered)
		Free ($\mu\text{g.}$)	Total ($\mu\text{g.}$)	Conjugated (%)	Free ($\mu\text{g.}$)	Total ($\mu\text{g.}$)	Conjugated (%)	Free ($\mu\text{g.}$)	Total ($\mu\text{g.}$)	Conjugated (%)			
After oral administration on grass													
5th instar	1690	532	592	10	260	324	20	32	84	62	60	62	16
	1670	220	224	2	68	88	23	40	84	53	63	28	17
	2510	1144	1144	0	220	252	13	20	48	58	61	60	4
	2470	576	600	4	208	236	12	52	64	19	59	39	7
Immature adult	400	60	64	6	84	100	16	Not measured, <80 $\mu\text{g.}/\text{locust}$			—	41	12
	700	112	148	24	96	160	40				—	44	32
	1140	260	312	17	140	176	21				—	43	18
	1140	92	92	0	124	146	15				—	21	9
	1560	328	328	0	116	140	17				—	30	5
	1840	888	912	3	276	324	15				—	67	6
	2930	672	752	11	236	284	17				—	35	12
Amount injected ($\mu\text{g.}$)													
After injection (grass diet)													
5th instar hoppers	560	252	304	17	48	64	25	No excreta†			—	66	19
	560	284	396	28	52	84	38				—	86	30
	560	228	300	24	36	72	50				—	67	29
	560	48	68	29	No excreta†			188	228	18	—	53	20
	560	28	28	0				92	180	49	—	37	42
	670	No excreta†						116	196	41	60	38	41
	670							104	116	10	30	22	10
	670							100	124	19	138	39	19
	670							No excreta†			96	128	25

* Hoppers moulted on 3rd and 4th days.

† Locust moulted.

Table 2. *Free and total sulphadimidine in excreta of immature adult locusts after injection of 850 $\mu\text{g.}$ of sulphadimidine (grass diet)*(All quantities in $\mu\text{g.}$)

1st day			2nd day			3rd-7th days			8th-15th days			Recovery (%)	Conjugates (as % of recovered)
Free	Total	Conjugated (%)	Free	Total	Conjugated (%)	Free	Total	Conjugated (%)	Free	Total	Conjugated (%)		
440	440	0	112	124	10	56	68	18	40	64	37	82	7
362	376	4	52	92	43	20	32	38	52	68	24	67	14
72	84	14	104	124	16	72	340	79	40	64	38	72	53
337	401	16	100	140	29	52	232	78	32	48	33	97	36
304	352	14	72	92	22	72	100	28	20	36	44	68	19

evaporation to dryness on the water bath, the extract was taken up in acetone and separated chromatographically on paper (Whatman no. 1).

The spots were developed by spraying with 1% salicylaldehyde in 25% (w/v) trichloroacetic acid which gave an immediate yellow spot with sulphadimidine. After heating at 120° for 30 min., both sulphadimidine and acetylsulphadimidine gave a brown spot with an intense yellow-green fluorescence in ultraviolet light.

Free and acetylated sulphadimidine were identified in the extract by comparison with the behaviour of the authentic materials run on the same paper (Table 3). The acetyl compound was present in the excreta of both immature adults and 5th instar hoppers on the first and second days after ingestion of sulphadimidine.

Acetylation of 6-amino-4-nitro-o-cresol in the locust. The compound, 0.66 mg. dissolved in 0.01 ml. 0.5N-HCl, was administered orally to two immature adults. This dose had no apparent ill effect, nor did the same quantity injected into the thorax of two other locusts. The excreta, collected 24 hr. later, were extracted with hot 80% (v/v) aqueous ethanol, filtered from grass residues, and the concentrated filtrate examined chromatographically on Whatman no. 1 paper for 6-acetamido-4-nitro-o-cresol and for the compound fed (cf. Smith, Smithies & Williams, 1953). The extract contained a substance having the same R_F values as 6-acetamido-4-nitro-o-cresol in benzene-acetic acid-water, 1:1:2 ($R_F=0.84$); benzene-*n*-butanol-ammonia sp.gr. 0.880, 2:5:2 ($R_F=0.44$), and *n*-butanol-acetic acid-water, 4:1:5 ($R_F=0.95$), and giving the same yellow colour when

sprayed with π -NaOH. Considerable amounts of unchanged amino compound were also detected on the chromatograms (R_F values, 0.56, 0.20 and 0.95, respectively).

Table 3. R_F values of sulphadimidine and acetylsulphadimidine

(Paper: Whatman no. 1; time of run, 4 hr.)

Solvent mixture (parts by volume)	R_F values	
	Sulphadimidine	Acetylsulphadimidine
isoPropyl ether-formic acid-water, 5:1:1	0.35	0.59
Ethyl methyl ketone-water, 1:1	0.97	0.86
Ethyl methyl ketone-2N-ammonia, 1:1	0.47	0.20

DISCUSSION

The results obtained show that the locust, in common with a wide selection of animal species (see Schnitker, 1948), can acetylate the aromatic amino group.

Absorption of sulphadimidine from the intestine appears to be slow and the high 'free' values found on oral administration are undoubtedly due to unabsorbed sulphadimidine. The degree of conjugation of that material which is absorbed is very variable, values ranging from 0 to 80% being obtained, without significant difference between hoppers and adults.

In some early experiments it was observed that the total recoveries of administered material were very low, particularly when hoppers were fed just before the moult. Examination of the moulted skin showed that it contained only a small amount of material diazotizable after hydrolysis and that the detoxication did not involve deposition of waste material in the exuviae as has sometimes been suggested (cf. Wigglesworth, 1950). It seems that the low recovery in these cases is to be associated

with the cessation of feeding near the moult; this allows the drug 2-3 days for absorption since no excreta are produced when the insects are not eating. The excretion of material once inside the locust is very slow and in prolonged experiments (cf. Table 2) the recovery is higher. Measurements were discontinued after 15 days, but even 24 days after feeding, weak diazo reactions were obtained on the excreta which indicated that about a third of the eliminated drug was conjugated.

After the acetylation of sulphadimidine had been demonstrated it seemed of interest to feed 6-amino-4-nitro-*o*-cresol since this is a major metabolite of the insecticide 4:6-dinitro-*o*-cresol (DNOC) in the rabbit. The former compound has a low toxicity to rabbits and the locusts tolerated doses of 0.66 mg. without ill effects, while DNOC has a LD_{50} in *Locusta* of about 0.02 mg./locust (Hopf, 1951). In the locust some of the material fed was further metabolized to its acetyl derivative as is the case in the rabbit (Smith *et al.* 1953).

SUMMARY

1. The ability of *Locusta migratoria* to acetylate an aromatic amino group has been examined.
2. A variable amount of sulphadimidine (2-(*p*-aminobenzenesulphonamido)-4:6-dimethylpyrimidine) is conjugated by the locust; N^4 -acetylsulphadimidine has been identified in the excreta by paper chromatography.
3. Sulphadimidine is poorly absorbed and slowly excreted by the locust.
4. Acetylation of 6-amino-4-nitro-*o*-cresol, a possible metabolite of 4:6-dinitro-*o*-cresol, also occurs in the locust.

The authors are grateful to Prof. R. T. Williams for his interest in this work and to the staff of the Anti-Locust Research Centre, British Museum (Natural History), for supplies of locusts.

REFERENCES

- Bratton, A. C. & Marshall, E. K. Jun. (1939). *J. biol. Chem.* **128**, 537.
- Brodie, B. B. (1952). *3rd Nat. Med. Chemistry Symposium, Amer. Chem. Soc. Abstr.* p. 80.
- Brown, A. W. A. (1951). *Insect Control by Chemicals*, p. 63. New York: Wiley.
- Chauvin, R. (1941). *Ann. Soc. ent. Fr.* **110**, 133.
- Ferguson, W. C. & Kearns, C. W. (1949). *J. econ. Ent.* **42**, 810.
- Hollande, M. A. (1909). *Arch. Univ. Grenoble*, **21**, 459.
- Hopf, H. S. (1951). *2nd Int. Congr. Pl. Prot.* Sections 1, 2, 4 and 6.
- Krebs, H. A., Sykes, W. O. & Bartley, W. C. (1947). *Biochem. J.* **41**, 622.
- Perry, A. S. & Hoskins, W. M. (1950). *Science*, **111**, 600.
- Schnitker, M. A. (1948). *The Sulphonamide Compounds in Treatment of Infections*, p. 51. New York: Oxford University Press.
- Shephard, H. H. (1939). *The Chemistry and Action of Insecticides*. New York: McGraw Hill.
- Smith, J. N., Smithies, R. H. & Williams, R. T. (1953). *Biochem. J.* **54**, 225.
- Smith, J. N. & Williams, R. T. (1948). *Biochem. J.* **42**, 351.
- Swingle, M. C. (1934). *J. econ. Ent.* **27**, 1101.
- Uvarov, B. P. (1948). *Anti-Locust Bull.* **1**, 1.
- Wigglesworth, V. B. (1950). *Principles of Insect Physiology*, 4th ed. pp. 33, 384. London: Methuen.
- Williams, R. T. (1947). *Detoxication Mechanisms*. London: Chapman and Hall.
- Williams, R. T. (1951). *Annu. Rev. Biochem.* **20**, 441.
- Winteringham, F. P. W., Loveday, P. M. & Harrison, A. (1951). *Nature, Lond.*, **167**, 106.
- Zukel, J. W. (1944). *J. econ. Ent.* **37**, 796.