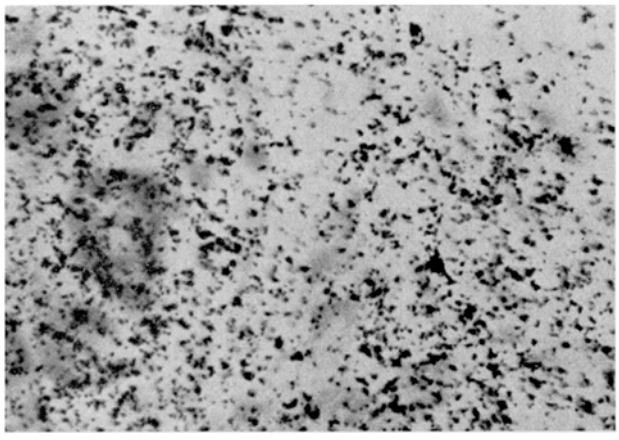

Fig. 5. – 24 h après l'injection; pose: 4 jours
La densité des grains augmente dans les neurones et davantage encore dans les fibres postganglionnaires

en évidence dans les protéines nerveuses[5,6], il reste à démontrer la réalité d'une migration des protéines élaborées par les cellules ganglionnaires[7].

J. VERNE et B. DROZ

Laboratoire d'histologie, Faculté de médecine de Paris, le 2 octobre 1959.

Summary

A migration of radio-activity is shown in the postganglionic nerves of the sympathetic ganglion, if an interval is allowed to elapse between the injection of Methionine ^{35}S and sacrifice. This process suggests a migration of proteins elaborated by the neurons.

[5] A. V. PALLADIN et N. M. POLJAKOWA, 4e Congr. Intern. Biochem., Vienne (1958), 2–12.
[6] H. WAELSCH, 4e Congr. Intern. Biochem., Vienne (1958), Sympos. III.
[7] S. OCHS et E. BURGER, Amer. J. Physiol. *194*, 499 (1958).

Contrasting Tail and other Responses to Morphine and Reserpine in Rats and Mice[1]

Introduction. The mouse tail reaction to morphine described by STRAUB[2] and HERMANN[3] has proved of value in pharmacological studies for assessing the effects and interaction of various drugs on the central nervous system, *vide* HOLTEN[4].

During work at present in progress we have observed in rats a tail reaction to Reserpine which seems to be the opposite to the STRAUB-HERMANN reaction to morphine in mice, and as this latter has been so useful to pharmacologists in the past, we feel that tail reactions of these rodents to Reserpine should be described and contrasted with that seen after morphine.

[1] This work was aided by a grant from the Medical Research Council.
[2] W. STRAUB, Dtsch. med. Wschr. *38*, 1462 (1911).
[3] O. HERMANN, Biochem. Z. *39*, 216 (1912).
[4] C. H. HOLTEN, Acta pharmacol. toxicol. *13*, 113 (1957).

Response to morphine. Four male albino rats weighing 110–410 g injected subcutaneously with 10 mg/kg morphine sulphate, showed a tail reaction within 30 min similar to, but less pronounced than, the STRAUB-HERMANN tail sign in mice. The normal rat carries its tail horizontally along the ground and if the tail is lifted up, it returns rapidly to the horizontal (Fig. 1). After morphine the tail tends to be slightly raised though still horizontal, but if lifted up remains for some time in an almost vertical position while the animal moves around (Fig. 2), and then slowly returns to the horizontal. That is to say, the tail is curled upwards and also lacks tone. Rats given morphine show no abnormal grasping reflex and their eyes, hair, and posture remain normal, but there is some increase in activity when the drug effect is at its maximum.

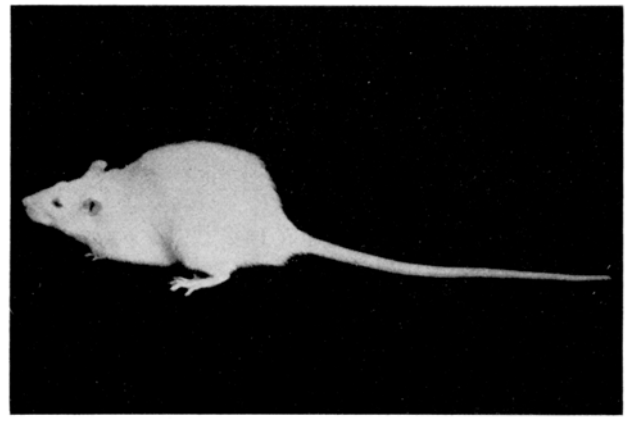

Fig. 1. – Normal rat R/1/11 weight 360 g

Fig. 2. – R/1/11 1 h after 10 mg/kg Morphine sulphate subcutaneously. Note curling up tail and open eyes

Response to Reserpine. Thirteen male albino rats weighing 130–420 g injected subcutaneously with 5 mg/kg Reserpine showed a tail reaction diametrically opposite to that seen after morphine. This appeared in some cases 3 or 4 h, but in others not for about 18 h, after injection, continued to be very marked for two days, and thereafter usually decreased. The response was quite unmistakable and recognised by a marked increase in tone in the tail, and by the fact that it was slapped down after being lifted to the vertical position and released. But the most striking phenomenon was seen when a finger was placed under the proximal portion of the tail and the tail lifted with a slow stroking movement. When this was done the tail curled down and round the finger with a grasping movement and remained flexed (Fig. 3).

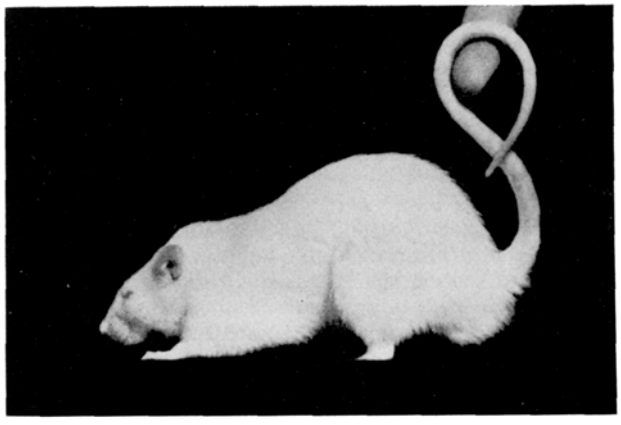

Fig. 3. – R/1/12 340 g, $4^1/_2$ h after 5 mg/kg Reserpine subcutaneously. Note down curling tail and closed eyes

This reaction can be facilitated during the onset and when the effect of the drug is diminishing, if the stimulus is applied repeatedly by stroking the length of the tail with a finger or pencil placed underneath it.

Concurrent with the tail reaction the rats also developed a very strong grasp reflex in all four paws, similar to that seen in monkeys after Reserpine. This reflex could be demonstrated by placing a thick wire or small screwdriver in the fore-paws and gently raising the rat until it was suspended (Fig. 4). During this stage some rats tended to use the mouth to hang on with, and to curl up and grasp the wire with all four paws. The Reserpine monkey will also do this latter and cling on to an arm extended horizontally with all four limbs, COLE and GLEES[5].

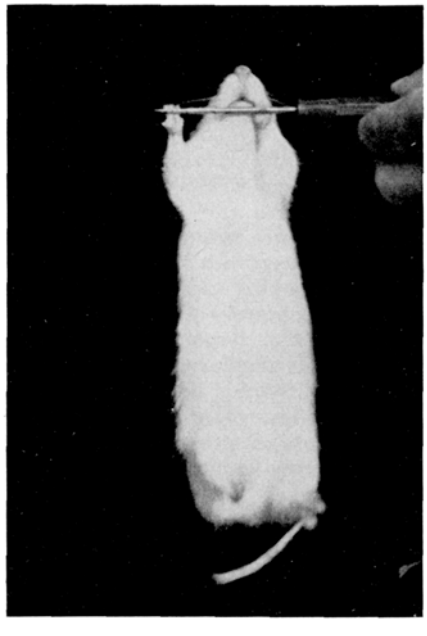

Fig. 4. – R/1/12 5 h after 5 mg/kg Reserpine subcutaneously showing strong grasp reflex

Although the duration of the tail and grasping reflexes varies in individuals, we have found that they tend to be more marked and to last longer in animals over 300 g. Other typical Reserpine phenomena observed include decrease in activity, piloerection, diarrhoea, closing of the eyes, tremor, abnormal and hunched posture.

[5] J. COLE and P. GLEES, Lancet *1*, 338 (1956).

Table I sets out the contrasting morphine and Reserpine effects.

Table I

Contrasting Morphine and Reserpine effects in rats

	10 mg/kg Morphine	5 mg/kg Reserpine
Tail	Curls up	Curls down
	Weak tone	Strong tone
Grasp reflex	Normal	Very strong
Eyes	Open	Closed
Piloerection	Absent	Present
Tremor	Absent	Present
Diarrhoea	Absent	Present
Activity	Increased	Decreased
Posture	Normal	Abnormal

Also, two rats showing the full Reserpine syndrome were given 10 mg of Methyl-phenidate (Ritalin) subcutaneously. Within 20 min the Reserpine signs had almost disappeared, but they reappeared 12 h later. This confirms the antagonism between Methyl-phenidate and Reserpine reported by COLE and GLEES in monkeys.

Tail responses to Morphine and Reserpine in mice. The typical STRAUB-HERMANN tail sign was observed in two mice given 10 mg/kg morphine subcutaneously for comparison with the rats. In ten mice given 5 mg/kg Reserpine subcutaneously, although the tendency for the tails to curl round the finger when lifted up was perhaps not quite so marked as in the rats, there was pronounced increase in tone and the tail was 'pressed' down on a flat surface, and would curl down over the edge of a table.

Mice in this condition given morphine of a dose up to 20 mg/kg held their tails at an angle of about 45°, but such tails unlike the true STRAUB-HERMANN tail tended to grasp when a finger was placed underneath them. This general observation made in mice for comparison with the tail reactions to Reserpine and morphine seen in our rats might indicate, as SCHNEIDER[6] suggets, that the Reserpine-morphine relationship is competitive at various levels of the central nervous system.

The careful study of TRIPOD *et al.*[7], in which they found that in mice Reserpine antagonised the morphine tail-sign but was synergistic with morphine on the pupil reaction, supports the view that these two drugs are competitive at various levels rather than directly antagonistic as Reserpine and Methyl-phenidate appears to be. In fact these authors state 'that a true antagonism is unlikely'.

Controls. We tested the tail and grasp reflexes of 50 normal albino rats of both sexes and varying weight from 25–580 g. The results are shown in Table II.

Both the grasp and tail signs found were little more than equivocal and in no case as pronounced as those seen after the drugs. The downward curling tails which needed much facilitation, had not as much tone, nor were they slapped down as was the case after Reserpine. The two up-curling tails were also doubtful; in fact can best be described as showing a tendency to resemble the morphine tail.

[6] J. A. SCHNEIDER, Proc. Soc. exp. Biol. Med. *87*, 614 (1954).

[7] J. TRIPOD, H. J. BEIN, and R. MEIER, Arch. int. Pharmacodyn. *96*, 406 (1954).

Table II

Incidence of tail and grasp reflexes in 50 normal rats resembling those seen after Morphine or Reserpine

Weight in g	Sex	No.	Tail down	Tail up	Grasp reflex
25–54	M	6	—	—	4
25–54	F	2	—	—	—
55–99	M	3	—	—	—
55–99	F	5	—	—	—
100–249	M	10	—	1	—
100–249	F	4	—	1	1
250–580	M	15	4	—	—
250–580	F	5	—	—	—

We are grateful to CIBA Laboratories for supplying Reserpine.

J. COLE and D. P. DEARNALEY

University Laboratory of Physiology and University Department of Biochemistry, Oxford, October 9, 1959.

Zusammenfassung

Charakteristisch verschiedene Schwanzstellungen zeigen Albino-Ratten und -Mäuse als Morphium- bzw. Reserpinreaktion. Verstärkte Schwanzreaktionen erhält man bei der Maus nach Morphium, bei der Ratte nach Reserpin. Methylphenidat erweist sich bei der Ratte antagonistisch gegen Reserpin, während im Mäuseversuch nach Reserpin injiziertes Morphium kompetitiv zu wirken scheint.

DISPUTANDUM

Lignin and the Formation of Wood

The paper by F. F. NORD and W. J. SCHUBERT on the title subject in Experientia *15*, 245 (1959) requires several amendments and supplementary comments. For the sake of brevity, the discussion here has been restricted to the grossest inadequacies and discrepancies.

The best preparation of wood lignin available at present is BJÖRKMAN's milled wood lignin (MWL)[1]. MWL prepared from spruce wood contains 0·3 phenolic hydroxyl groups per C_6–C_3 unit, whereas the minute fraction of ligninlike material which is extractable from sawdust according to F. BRAUNS contains twice this amount of phenolic hydroxyl[2,3a]. Here the latter preparation was extracted from wood using acetone in preference to alcohol and then separated from lignans and other admixtures by countercurrent distribution. NORD and SCHUBERT claim that 'enzymically liberated lignin' is identical with BRAUNS' 'native lignin'. This implies therefore that it is not identcal with the main part of *true* wood lignin or protolignin. Thus, for instance, for Scots pine, values of 815 and 695 were found by NORD *et al.*[4] for the molecular weights of native and enzymically liberated lignin respectively. Spruce MWL on the other hand, although probably partially degraded by milling, has a molecular weight of 11,000[1], in the same range as that of FREUDENBERG's biosynthetic lignin[3b].

BROWN and NEISH[5] were the first to show that radioactive shikimic acid introduced into plants is incorporated into lignin and that radioactive vanillin etc. is formed on oxidation of such lignin. Independently, EBERHARDT and NORD[6a] confirmed this in more elaborate experiments. The role of shikimic acid in lignin formation is just the same as in all other cases of biosynthesis of C_6–C_3 substances. Various American authors have shown that shikimic acid is transformed into prephenic acid and thence into phenylpyruvic acid or cinnamic acid or phenylalanine. As FREUDENBERG has shown, labelled phenylalanine is transformed by spruce saplings either in the needles or in the cambium or en route from the needles to the cambium into coniferin, which was isolated in radioactive form from cambial sap[7].

In conifers coniferin and in other plants corresponding glucosides in addition assume the cardinal position in lignin formation. These are the immediate starting materials for lignification, as has been demonstrated by FREUDENBERG *et al.*[8] in the case of spruce lignin using radioactive coniferin.

NORD, SCHUBERT and ACERBO[9] have shown that radioactive *p*-hydroxyphenylpyruvic acid is incorporated into sugar cane lignin and that such lignin forms radioactive aldehydes on oxidative degradation. This important observation does not indicate however that *p*-hydroxyphenylpyruvic acid is the only or even an essential starting material for lignin formation, as NORD and SCHUBERT suggest. In an analogous experiment carried out with labelled ferulic acid, FREUDENBERG[10] found that lignin is formed which yields radioactive ketones on ethanolysis according to HIBBERT. The correct interpretation of this data is that phenylpyruvic acid or cinnamic acid or phenylalanine is oxidized on the way leading to coniferin in the 4-position and in the 3-position, which is also methylated. The acids are reduced at the carboxyl group, are converted into unsaturated compounds and finally glucosidized. The absolute sequence of these operations has not yet been definitely established[11]; *p*-hydroxyphenylpyruvic acid and ferulic acid may be essential intermediates in these transformations or may merely be transformed independently in side reactions into coniferin and other glucosides. The role of *p*-hydroxyphenylpyruvic acid is rather uncertain, as it has been shown that its incorporation into conifer lignin proceeds rather unsatisfactorily[11,12].

[1] A. BJÖRKMAN, Svensk Papperstidning *59*, 477 (1956).

[2] G. AULIN-ERDTMAN and L. HEGBOM, Svensk Papperstidning *61*, 187 (1958).

[3] a) K. FREUDENBERG, in *Biochemistry of Wood*, Proc. 4th Int. Congr. Biochem., Vol. 2, p. 125 (1959). – b) K. FREUDENBERG and K. DALL, Angew. Chem. *42*, 606 (1955).

[4] G. DESTEVENS and F. F. NORD, Fortschr. chem. Forsch. *3*, 95 (1954).

[5] S. A. BROWN and A. C. NEISH, Nature *175*, 688 (1955).

[6] a) G. EBERHARDT and F. F. NORD, Arch. Biochem. Biophys. *55*, 578 (1955). – b) G. EBERHARDT and W. J. SCHUBERT, J. Amer. chem. Soc. *78*, 2835 (1956).

[7] K. FREUDENBERG and F. NIEDERCORN, Chem. Ber. *91*, 591 (1958).

[8] K. FREUDENBERG, H. REZNIK, W. FUCHS, and M. REICHERT, Naturwiss. *42*, 29 (1955).

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[10] K. FREUDENBERG, Angew. Chemie *68*, 92, 511 (1956). – S. A. BROWN and A. C. NEISH, Canad. J. Biochem. Physiol. *34*, 769 (1956).

[11] S. A. BROWN, D. WRIGHT, and A. C. NEISH, Canad. J. Biochem. Physiol. *37*, 25 (1959).

[12] G. BILLEK, in *Biochemistry of Wood*, 4th Int. Congr. Biochem., Vol. 2, p. 211 (1958).