Brit. J. Pharmacol. (1949), 4, 98.

THE PHARMACOLOGY OF THE OPTICAL ISOMERS OF AMIDONE (2-DIMETHYLAMINO-4 : 4-DIPHENYLHEPTAN-5-ONE)

BY

R. H. THORP

From the Wellcome Physiological Research Laboratories, Beckenham, Kent

(Received September 22, 1948)

A brief description of the preparation of the optical isomers of amidone has already been published (Thorp *et al.*, 1947b), in which it was shown that it is possible to prepare these substances by resolution of 1 : 1 - diphenyl - 3 - dimethylamino-valeronitrile, the penultimate compound in the synthesis of amidone, and conversion of the optically active nitriles into the corresponding ketones. Some brief pharmacological results were given in that note, but it is now possible to describe the pharmacology of these substances more fully.

Analgesic activity

The relative analgesic activities of *d*-, *l*-, and *dl*-amidone were determined in comparison with that of morphine. The method used has already been described by the author (1946) ; it was a modification of the radiant heat stimulation method of Hardy, Wolff, and Goodell (1940). The comparison was performed on a group of forty rats on each of four days, and two dose levels for each drug were used. The animals were randomly arranged into eight groups of five rats, and the treatments changed on each day so that all rats had received one dose of each drug at the completion of the test. The initial pain threshold was determined before injection ; the drugs, dissolved in amounts of saline such that the appropriate doses were contained in 0.5 ml. per 100 g. of rat, were injected subcutaneously at intervals of 2 min. This interval was introduced in order to allow for the time taken in estimating the pain threshold subsequent to injection, so that responses might be measured at equal intervals.

Previous tests had indicated that the effects of morphine and amidone upon the pain threshold of rats are maximal approximately 30 min. after injection and that the curves for the duration of action of the two substances are almost identical. A single determination of the pain threshold half an hour after injection was therefore used for estimations of analgesic potency.

Table I shows the results obtained in the comparison of the analgesic activities of *d*-, *l*-, and *dl*-amidone with that of morphine.

The equiactive doses in the sixth column were obtained by estimating the dose of each drug producing a 50 per cent increase in pain threshold,

TABLE I

THE COMPARATIVE ANALGESIC ACTIVITIES OF THE OPTICAL ISOMERS OF AMIDONE BY THE RAT METHOD

Drug tested	Dose mg./kg.	No. of rats	Mean increase in pain threshold			Equiactive doses mg./kg. (±%S.E.)	Activity ratio (morphine = 1)
			Per cent	Standard error	Slope		
d-Amidone hydrochloride	10 20	20 20	34.9 34.8	3.45 3.65	nil	—	—
l-Amidone hydrochloride	0.8 1.2	20 20	31.9 61.8	3.19 5.75	169.8	1.02 ±10.45%	2.1
dl-Amidone hydrochloride	1.2 2.4	20 20	41.0 63.5	3.78 8.80	74.8	1.58 ±12.05%	1.36
Morphine sulphate	2.0 3.0	20 20	46.8 63.7	4.72 10.01	96.0	2.16 ±11.15%	1.0

the slope value estimated for each regression line being used.

The activity ratios are the ratios of the reciprocals of the equiactive doses taking the value for morphine as unity. The three values for the slopes were tested for similarity, and it can be shown that, for $P = 0.95$, the differences between them are not statistically significant. The standard errors of the equiactive doses in the fifth column do not preclude the possibility that *l*-amidone is twice as active as the racemic isomer.

The results show that in the rat *dl*-amidone hydrochloride is probably a rather more powerful analgesic drug than morphine sulphate, and that the activity of the *laevo* isomer is very much greater than that of the racemic form.

The *dextro* isomer failed to produce a graded increase in pain threshold when given in large doses, and the observed rise in pain threshold was probably not due to true analgesic action. In doses comparable with those of *dl*-amidone, *d*-amidone is without effect upon the pain threshold in rats. It is therefore very probable that the *laevo* isomer is twice as active as the racemic form and the *dextro* compound inactive.

Toxicity

The toxicity of the optical isomers of amidone was determined by intravenous injection into mice, the method of calculating the LD50 value described by Kärber (1931) being used. The doses were always given in 0.25 ml. of physiological saline and the results obtained are given in Table II.

It will be seen that the LD50 values for the three isomers are of the same order of magnitude. The actual values obtained do not, however, agree with those previously reported by the author (1947a ; 1947b), which were between 10 and 18 mg./kg. We have repeated acute toxicity experiments on mice of two different strains and upon different days, and found that the absolute values of the LD50 for amidone isomers are subject to wide variations, although the results are comparable within individual experiments.

Finnegan *et al.* (1948) found the LD50 of *dl*-amidone hydrochloride to be 9.2 ± 0.4 mg./kg. in rats upon intravenous injection, but pointed out that the toxicity in acute experiments is very much greater than that of morphine, whereas the values obtained with smaller doses in a subacute experiment were much more similar.

These findings show that the acute toxicity of amidone is not due to the central nervous depressant property since this is almost absent in the *dextro* isomer. The acute toxic effect is the result of sudden cardiovascular failure, and the LD50 value is determined largely by the resistance of the animals to a critical fall of blood pressure of brief duration

There is evidence, however, from the results given above that the toxicity of *l*- and *dl*-amidone is enhanced by the depressant action these drugs exhibit since the LD50 value for *d*-amidone is just significantly greater than that of the other isomers.

When cats and dogs were anaesthetized with pentobarbitone sodium and arranged for recording blood pressure and respiration, the following results were obtained.

d-*Amidone.*—In both dogs and cats similar effects were observed after intravenous injections of *d*-amidone. Small doses of 1.0 or 2.0 mg./kg. caused a brief fall in blood pressure of 40–80

TABLE II

THE ACUTE TOXICITIES OF THE OPTICAL ISOMERS OF AMIDONE UPON INTRAVENOUS INJECTION INTO MICE

d-Amidone HCl		*l*-Amidone HCl		*dl*-Amidone HCl	
Dose mg./kg.	Mortality	Dose mg./kg.	Mortality	Dose mg./kg.	Mortality
22.64	0/20	11.32	0/20	8.00	0/20
32.00	14/20	16.00	2/20	11.32	2/20
45.28	20/20	22.64	9/20	16.00	3/20
		32.00	14/20	22.64	8/20
		45.28	17/20	32.00	14/20
		64.00	20/20	45.28	20/20
LD50 = 29.9 mg./kg. Fiducial limits (P = 0.95) 27.47 to 32.58		LD50 = 26.02 mg./kg. Fiducial limits (P = 0.95) 22.89 to 29.55		LD50 = 24.24 mg./kg. Fiducial limits (P = 0.95) 21.16 to 27.76	

mm. Hg. There was no depression of the respiration and when a series of doses of 2 mg./kg. were given at 15-min. intervals a similar fall in blood pressure was produced with each injection. The duration of each depressor response was from 1 to 2 min. Death resulted in all animals after the injection of 7–10 mg./kg. of *d*-amidone and was characterized by a precipitate fall of blood pressure to approximately one-third of the normal level. This effect was associated with temporary respiratory arrest followed by Cheyne-Stokes respiration, and death usually occurred in 20 min. or less. These effects were not modified by section of both vagi in dogs.

When guinea-pigs in which the brain and upper part of the spinal cord had been destroyed were supplied with artificial respiration and arranged for recording blood pressure, injections of 1 mg./kg. of *d*-amidone caused a marked fall in blood pressure similar to that observed in anaesthetized dogs or guinea-pigs.

These experiments show that the principal cause of the toxic action of *d*-amidone is a precipitate fall in blood pressure which is due to direct action upon the cardiovascular system.

l-*Amidone*.—The injection of *l*-amidone into the anaesthetized dog or cat caused effects which were markedly different from those described above. In the dog a first injection of 1 mg./kg. of *l*-amidone caused a fall of blood pressure of approximately 50 mm. Hg lasting about 1½ min. The blood pressure then rose to a value slightly above the normal. The respiration showed marked depression and became irregular. A second dose of 1 mg./kg. caused a much smaller fall in blood pressure followed almost at once by a prolonged increase of approximately 30 mm. Hg above the normal level. The respiration showed a further degree of depression. A third similar dose 10 min. later caused no detectable fall in blood pressure, but a further rise in the general level. After both vagus nerves were cut and the animal left for an interval of an hour, injections of 1 and 2 mg./kg. of *l*-amidone caused only a fall in blood pressure.

In the cat the blood pressure showed the same brief fall upon injection of the drug, followed by a return to a level slightly below the normal value. Subsequent doses caused a progressively less marked initial fall, but a general lowering of the level of blood pressure followed.

This initial fall in blood pressure has been examined by Shideman and Johnson (1948), who have shown that it becomes less upon subsequent injections and is a phenomenon exhibited by most analgesic drugs. They have termed the effect "acute vascular tolerance."

The effects of *l*-amidone were in general indistinguishable from those of *dl*-amidone described by Scott and Chen (1946), and, in our experiments upon animals in which the vagi were intact, cardiac slowing was also observed after injections of *l*-amidone.

The acute effects of the amidone isomers appear to be due to two principal actions: an immediate fall in blood pressure caused by direct action upon the heart and possibly also the vascular system, and, with the *l*- or *dl*-isomers, an additional depression of respiration and some slight degree of parasympathetic stimulation.

Effect upon respiration

The effect upon respiration was compared in the rabbit by means of the respiration recorder described by Gaddum (1941). The recorder was

TABLE III

THE EFFECT OF THE OPTICAL ISOMERS OF AMIDONE UPON THE RESPIRATORY MINUTE VOLUME IN RABBITS

d-Amidone HCl			*l*-Amidone HCl			*dl*-Amidone HCl		
Dose mg./kg.	Max. change in respiration per cent	Mean	Dose mg./kg.	Max. change in respiration per cent	Mean	Dose mg./kg.	Max. change in respiration per cent	Mean
1.5	+15	+19.5	1.4	−46	−37.3	2.8	−55	−56.5
1.5	+24		1.4	−32		2.8	−64	
2.5	+3		1.4	−34		2.8	−60	
2.5	−10	+4.7	1.7	−47	−51.2	2.8	−47	
2.5	+14		1.7	−53		3.4	−66	−61.5
2.5	+12		1.7	−53		3.4	−55	
5.0	−5	−5.0	2.5	−87	−89.0	3.4	−47	
			2.5	−91		3.4	−78	
						5.0	−94	−95.0
						5.0	−96	

connected to a rubber mask with inspiratory and expiratory valves and fitted to unanaesthetized rabbits restrained in a prone posture by broad cotton tapes. Since analgesic compounds commonly cause a marked fall in body temperature the animals were placed in a ventilated air thermostat maintained at 24° C. The recorder was of the differential type described in Gaddum's paper with tambours 4 in. in diameter. In these circumstances the record obtained was linearly proportional to the respiratory minute volume from a recorded height of 2 to 12.5 cm. upon the kymograph. Four to eight rabbits were used for the comparison of each compound with morphine as the "standard," and the peak percentage of respiratory depression was measured.

Both *dl*- and *l*-amidone depress the rabbit respiration to a marked degree when doses are given slightly in excess of those required to produce analgesia.

Table III shows the percentage change of the normal respiratory minute volume of rabbits measured at the point of maximum respiratory depression on the continuous records obtained with Gaddum's respiration recorder.

The results are expressed graphically in Fig. 1, from which it will be seen that the depressant effect of *l*-amidone upon the rabbit respiration is twice as great as that of the racemic compound, the effect of the *dextro* isomer being negligible in comparable doses. Some degree of stimulation is possibly caused by the latter drug.

The respiratory depressant property parallels the occurrence of analgesic properties in these three compounds and is therefore not the reason for the high toxicity of all three isomers.

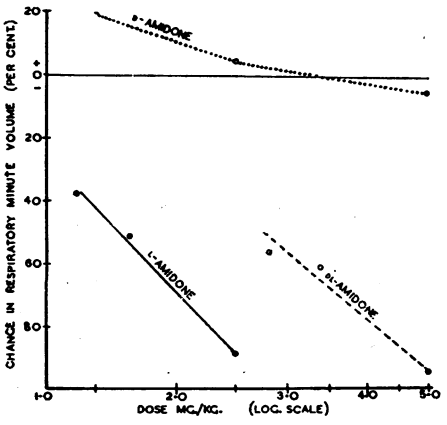

FIG. 1.—The effect of the optical isomers of amidone upon the respiratory minute volume of rabbits.

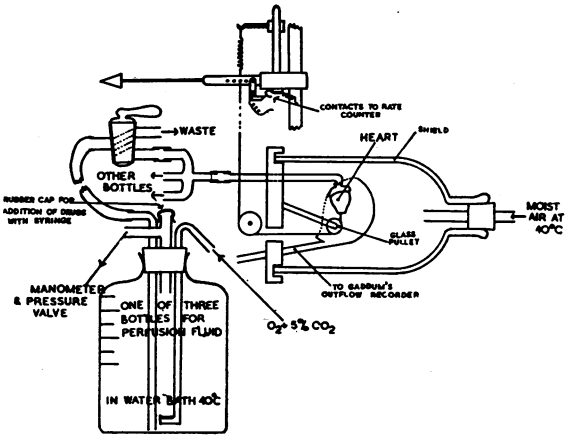

FIG. 2.—Apparatus for the perfusion of the isolated rabbit heart which enables the effect of accurately measured concentrations of drugs to be investigated.

Effect upon the isolated rabbit heart

The effect upon the isolated rabbit heart was estimated by means of the Langendorff preparation, but a modified apparatus was used which enabled the heart to be exposed to accurately known concentrations of the drugs.

This apparatus is shown diagrammatically in Fig. 2 and consists of a rigid vertical board through which the aortic cannula is mounted. This cannula can be connected to any one of three bottles of 1 litre capacity by three two-way stopcocks. The bottles, filled with Ringer solution, are kept at 39° C. in a water-bath. By this means the drug under test can be administered in known dilution from one of the bottles, and, after the rubber tubing connecting the cannula has been flushed out by means of the two-way tap, this solution can be substituted for the Ringer solution already flowing through the heart.

The outflow from the coronary vessels is recorded by means of a funnel beneath the heart carrying the perfusate to a Gaddum outflow recorder. The heartbeat is recorded by a thread from the apex of the left ventricle passing over a glass pulley and through a hole in the board to a lever on the kymograph. A record of the heart rate is made simultaneously by counting the closing of a pair of springy contacts mounted at the hinge of the recording lever. These contacts connect to a valve relay, and an impulse counter, described by the author (1948), is used to make the final record. By this means changes in coronary flow, heart rate, and the amplitude of contraction with each beat are recorded simultaneously.

The data shown in Table IV were obtained by means of the method described above. Results

TABLE IV

THE EFFECT OF THE OPTICAL ISOMERS OF AMIDONE AND OTHER ANALGESIC DRUGS UPON THE LANGENDORFF PREPARATION OF THE ISOLATED RABBIT HEART

Drug	Dilution	Effect upon the isolated rabbit heart			
		Coronary flow	Heart rate	Amplitude of beat	Notes
d-Amidone HCl	1 : 500,000 1 : 100,000	Nil +25%	Nil Slowed	Decreased Greatly decreased	Toxic conc.
l-Amidone HCl	1 : 500,000 1 : 100,000	+20% +30%	Nil Slight slowing	Decreased Greatly decreased	Toxic conc.
dl-Amidone HCl	1 : 500,000 1 : 100,000	+10% +20%	Nil Slowed	Decreased Greatly decreased	Toxic conc.
Morphine sulphate ..	1 : 100,000 1 : 50,000	Nil Nil	Nil Nil	Nil Nil	Non-toxic
Pethidine ..	1 : 100,000 1 : 20,000	Nil +10%	Nil Nil	Decreased Greatly decreased	Toxic conc.

obtained with morphine and pethidine were included for comparison.

It will be seen that all three isomers of amidone behaved similarly upon the heart. The toxic level was similar with each isomer and was found at concentrations of 1 : 100,000 and stronger. If it be assumed that the drug is distributed uniformly upon intravenous injection this level corresponds to a dose of the order of 10 mg./kg., a level comparable with that expected from the toxicity determinations.

Pethidine also proved to be toxic to the heart, but the concentration required was five times as great as that of amidone isomers ; this finding again agrees with the acute toxicity ratios for these drugs. Morphine, which is of low toxicity in mice and rats, was without cardiotoxic action at concentrations of 1 : 20,000 and less.

The principal toxic action of amidone isomers is therefore upon cardiac muscle, but it is increased with the *l*- and *dl*-isomers by the central nervous depression which these two drugs produce. The apparent stimulation of respiration shown by *d*-amidone is probably real and secondary to the fall of blood pressure produced by the action upon the heart of small doses.

Spasmolytic activity

Segments of rabbit ileum, caused to contract with a concentration of 1 in 4,000,000 carbamylcholine, showed approximately 70 per cent relaxation of the spasm after the addition of any one of the three amidone isomers in a concentration of 1 in 150,000 in the isolated organ bath. When 1 in 5,000 barium chloride was used in the same way each of the amidone isomers caused similar relaxation in a concentration of 1 in 120,000.

Pethidine was tested in the same way for purposes of comparison ; after carbamylcholine it had a similar spasmolytic effect in a concentration of 1 in 70,000 and after barium chloride the same concentration was effective.

These results show that amidone and its optical isomers are rather more active as spasmolytic drugs than pethidine. This effect is not associated with the optically active carbon atom but is a function of the molecule as a whole ; the action on the isolated heart already described is equally independent of the stereochemical configuration. Probably they act directly upon the muscle cells.

Local anaesthetic action

The local anaesthetic activity of the three isomers was compared with that of procaine by the intracutaneous weal method described by Bülbring and Wajda (1945). Eighteen to 24 guinea-pigs were used for each drug with two intracutaneous weals on each animal. Each animal received one weal from procaine and one weal from one of the test compounds. In half of the group of guinea-pigs high doses of procaine or of the amidone isomer were placed anteriorly and low doses posteriorly, and in the other animals the arrangement was reversed ; this was done in order to compensate for the slightly greater sensitivity of the skin in the anterior half of the body.

The results were assessed by calculating the mean slope of the dose response curves for

procaine and the amidone isomers, and thereby obtaining the potency ratio for the various drugs. A weighted mean potency ratio was then calculated for the group of animals treated with the same compound.

TABLE V

A COMPARISON OF THE LOCAL ANAESTHETIC PROPERTIES OF THE OPTICAL ISOMERS OF AMIDONE USING THE METHOD OF BÜLBRING AND WAJDA

The volume of solution injected for each dose was 0.1 ml.

Drug	No. animals	Conc. of drug mg./ml.	Mean potency ratio (procaine = 1)
d-Amidone hydrochloride	18	4.0 2.0	0.58
l-Amidone hydrochloride	24	2.0 1.0	3.51
dl-Amidone hydrochloride	24	2.0 1.0	2.14

The results are given in Table V from which it will be seen that local anaesthetic action is shown by all three isomers and is greatest in the *laevo* form. The potency of the racemic compound is the mean of those of the *d*- and *l*-isomers. Consequently the optical isomerism of amidone greatly influences the local anaesthetic activity, which is 3.5 times as great as procaine for the *laevo* isomer, but that this is not the only factor influencing this activity is shown by its occurrence with all three isomers.

Effect upon body temperature

The effect of these amidone isomers upon body temperature was determined in rabbits, six animals being used for each experiment. The temperatures were measured by rectal thermocouples connected to a Tinsley amplifier through a selector switch connecting each rabbit to the recorder once in 2 min. The final record was taken by means of a Siemen's six-colour chart recorder. The doses were given subcutaneously in a volume of 1 ml. physiological saline.

Groups of six rabbits were used together at one time, but each drug was given to more than one such group since some rabbits occasionally showed no effect at all from the drug. This absence of effect has previously been recognized in rabbits being used for testing pyrogenic substances, and a preliminary sorting test is usually employed to select animals of comparable sensitivity. Rabbits which showed no effect with the active drugs in these experiments were not included in the results. The remaining animals tended to fall into two groups, the larger one of which exhibited consistent changes in body temperature to the same treatment and a second smaller group of very sensitive animals which showed a very much more marked depression of temperature. These more sensitive animals numbered about 15 per cent of the total used. For the purposes of these comparisons all animals showing a fall in temperature were used for the calculation of the mean curves reproduced in Fig. 3.

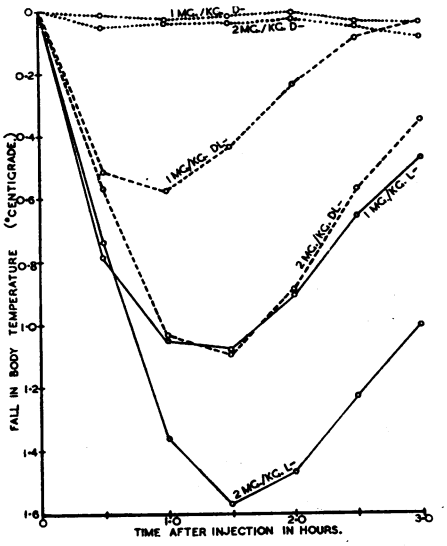

FIG. 3.—The effect of optical isomers of amidone upon the body temperature of rabbits.

The curves show that *d*-amidone had no effect upon body temperature, *l*-amidone produced the most marked depression, and the racemic isomer produced an effect which was very nearly half that of the *l*-isomer.

DISCUSSION

The central depressant actions of amidone are associated only with the *laevo* isomer. Consequently the racemic compound is approximately half as active on the central nervous system. The effects of amidone are complicated by the fact that some structural features other than the stereochemical configuration confer on the molecule marked toxic properties to the muscle cell, since these are also displayed by the *dextro* isomer.

The action of amidone upon the cardiovascular system has been examined by Scott and his colleagues (1947) using the cross-circulation technique of Heymans in dogs. They have shown that

the direct hypotensive effect was confined to the donor dog and was not the result of vagal mediation, and they concluded, because of the slowing of the heart of the recipient dog, that amidone was a stimulant of central parasympathetic nuclei, although the drug does not show any other characteristic parasympathetic effects.

The acute toxicity of amidone is almost entirely due to the direct effect on the heart whereas this type of effect is very small with morphine; this explains the findings of Finnegan *et al.* (1948) that morphine is far less toxic in acute experiments but that in chronic experiments with smaller doses there is less difference between this drug and amidone.

Compounds of the amidone type commonly show powerful local anaesthetic activity, and it is curious that among the effects which are influenced by the stereochemical configuration this was the only one in which the *dextro* isomer had a considerable degree of activity.

Summary

1. The *dextro, laevo,* and racemic optical isomers of amidone (2-dimethylamino-4 : 4-diphenylheptan-5-one) have been examined pharmacologically. The effects upon the central nervous system in mammals are associated with the *laevo,* and consequently also the racemic form.

2. The site of action of the acute toxicity of amidone was found to be upon the cardiac muscle cells. All three isomers of amidone were approximately equally toxic.

3. Spasmolytic activity was shown to be a function of the general structure of amidone and not associated with optical isomerism.

4. Local anaesthetic activity occurs in all three isomers, but is influenced by optical isomerism and is greatest in the *laevo* form.

5. The recently reported property of analgesic drugs, of producing a state of "acute vascular tolerance" to the depressor action resulting from intravenous injection, has been confirmed with *l*-amidone.

My thanks are due to Messrs. J. B. Attlesey, D. R. Howard, and R. F. Sellar for technical assistance. In addition I should like to thank Dr. E. Walton, of the Wellcome Chemical Research Laboratories, and Mr. P. Ofner, of the Wellcome Chemical Works, for the separation and provision of supplies of the optical isomers of amidone.

References

Bülbring, E., and Wajda, I. (1945). *J. Pharmacol.*, **85**, 78.
Finnegan, J. K., Haag, H. B., Larson, P. S., and Dreyfuss M. L. (1948). *J. Pharmacol.*, **92**, 269.
Gaddum, J. H. (1941). *J. Physiol.*, **99**, 257.
Hardy, J. D., Wolff, H. G., and Goodell, H. (1940). *J. clin. Invest.*, **19**, 649.
Kärber, G. (1931). *Arch. exp. Path. Pharmak.*, **162**, 480.
Scott, C. C., and Chen, K. K. (1946). *J. Pharmacol.*, **87**, 63.
Scott, C. C., and Chen, K. K., Kohlsteadt, K. G., Robbins, E. B., and Israel, F. W. (1947). *J. Pharmacol.*, **91**, 147.
Shideman, F. E., and Johnson, H. T. (1948). *J. Pharmacol.*, **92**, 414.
Thorp, R. H. (1946). *Brit. J. Pharmacol.*, **1**, 113.
Thorp, R. H. (1948). *Brit. J. Pharmacol.*, **3**, 271.
Thorp, R. H., Walton, E., and Ofner, P. (1947a). *Nature*, **159**, 679.
Thorp, R. H., Walton, E., and Ofner, P. (1947b). *Nature*, **160**, 605.