Brit. J. Pharmacol. (1951), **6**, 682.

THE ACTION OF DRUGS ON ISOLATED HUMAN BRONCHIAL CHAINS

BY

D. F. HAWKINS AND H. O. SCHILD

From the Department of Pharmacology, University College, London

(Received July 24, 1951)

In 1937, Sollmann and Gilbert observed under a microscope thin slices cut from human lungs obtained *post mortem* and from human foetal lung, and found that histamine and acetylcholine constricted the bronchi whereas adrenaline dilated them.

Recently, Castillo and de Beer (1947) introduced the use of an isolated bronchial muscle preparation consisting of a chain of guinea-pig tracheal rings. We have applied the same principle to the study of drug action on human bronchi, using a chain of bronchial rings. This preparation gives reproducible results and is suitable for quantitative work, and it has been used to study the activity of several broncho-constrictor and bronchodilator drugs and the antagonism between mepyramine and histamine. A similar preparation has recently been described in a preliminary communication by Rosa (1951).

METHODS

Source of material.—Most of the work was performed on pieces of lung removed at operation from patients with carcinoma or bronchiectasis.* In lung carcinoma, the tumour is often relatively localized, and it is then possible to dissect normal bronchi from unaffected parts of the lung. In the specimens of bronchiectatic lung we have had, the disease was usually more widespread, but one or more normal bronchi could generally be found. The surgeon was sometimes able to give guidance on this point.

Post-mortem material is not easy to obtain fresh, but on occasion lungs were received from fatal accident cases within one or two hours of death. A further source of material is human foetus from therapeutic abortions; with this material preparations can be made from the trachea and primary bronchi.

No difference in response to drugs was observed between material from these various sources. Some preparations deliberately made from bronchiectatic bronchi appeared to respond normally to drugs. Preparations from the bronchial muscle of pigs suffering from various forms of pulmonary disease were investigated by Macht and Ting (1921); they did not describe any abnormal responses to drugs, although they found reduction or absence of sensitivity in preparations from severely diseased animals.

Muscle strips from primary bronchi.—On one occasion muscle strips dissected from the primary bronchus of a human lung were examined. They were found to respond to drugs, but the responses soon decreased in size and had ceased altogether two or three hours after the preparations had been set up.

* We are grateful to Professor R. S. Pilcher and to Dr. H. Herxheimer, of the Surgical Unit, University College Hospital, for making the material available to us.

Experimental procedure.—On removal from the patient the lobe or lung was placed in cold Ringer solution and transferred to the laboratory. Suitable bronchi were dissected out and cut into rings, which were tied in chains with loops of cotton. The chains were suspended in Ringer solution at 37° C. aerated with oxygen through a fine sintered glass plate. Responses were recorded with a light balsawood frontal writing lever on a lightly smoked kymograph drum rotating at 1 mm. per minute. The tension on the lever was 200 mg. and the magnification × 12. All sizes, from secondary bronchi (lumen diameter 6–8 mm.) to fifth order bronchi (diameter about 1 mm.), were found to be suitable. In general three to five rings were adequate to give sufficiently large recorded responses for quantitative work, since the smaller bronchi contain progressively less cartilage and are capable of a proportionately greater degree of contraction.

Drugs were added to the bath or occasionally introduced dissolved in the Ringer solution. When the response to a dose had reached a steady level, the bath fluid was replaced every five minutes with fresh pre-warmed and pre-aerated Ringer solution until the preparation had recovered its previous tonus level. An automatic apparatus, modified from that described by Schild (1947), was utilized for this purpose when several preparations were being used simultaneously. The Ringer solution used in some of the experiments was that described by Krebs and Henseleit (1932), aerated with $O_2 + 5\%$ CO_2. A similar solution with one-tenth of the concentration of sodium bicarbonate, and aerated with O_2 alone, was used in the remainder of the experiments. Both solutions had a *p*H lying between 7.1 and 7.4 under the experimental conditions, and there was no appreciable difference between the behaviour of preparations in them.

Under these conditions the chains maintained a steady baseline and gave repeated steady or graded responses to bronchoconstrictor or bronchodilator drugs for 10 to 15 hours. In contrast we have found that preparations maintained in Tyrode's fluid, aerated with oxygen at 37° C., become insensitive and cease to give responses within an hour or two. The bronchi still respond to drugs when used after preservation in Ringer solution at 4° C. for two or three days.

The sympathomimetics were compared with synthetic *l*-adrenaline *B.P.* (Burroughs Wellcome & Co.) made up as hydrochloride. The other drugs used were *dl*-noradrenaline hydrochloride (Winthrop Stanley), *dl-iso*propylnoradrenaline sulphate (Burroughs Wellcome), *l*-ephedrine hydrochloride *B.P.* (Burroughs Wellcome), and aminophylline (Burroughs Wellcome). Fresh dilutions of adrenaline, noradrenaline, and *iso*propylnoradrenaline were made up every two or three hours in distilled water containing ascorbic acid (10^{-5}).

Drug concentrations are given in terms of the active base. The bath volume in all the Figures was 50 c.c.

RESULTS

Action of parasympathomimetic drugs

Acetylcholine added to the bath causes the chains to contract. A contraction of 50 per cent of the maximum is obtained with concentrations of the order of 10^{-6}. Fig. 1*a* shows that the effects were repeatable and that graded contractions could be obtained. The responses are readily reversed on changing the bath fluid. Fig. 1*b* shows reductions of the response to acetylcholine by atropine in concentrations 1 : 2,500,000,000 and 1 : 500,000,000.

Pilocarpine and carbachol also contract the chains. Pilocarpine has about four times the activity of acetylcholine ; carbachol is also more active than acetylcholine. The responses to these drugs take longer to reach a steady level than those due to acetylcholine ; they also take longer to reverse when the bath is washed out.

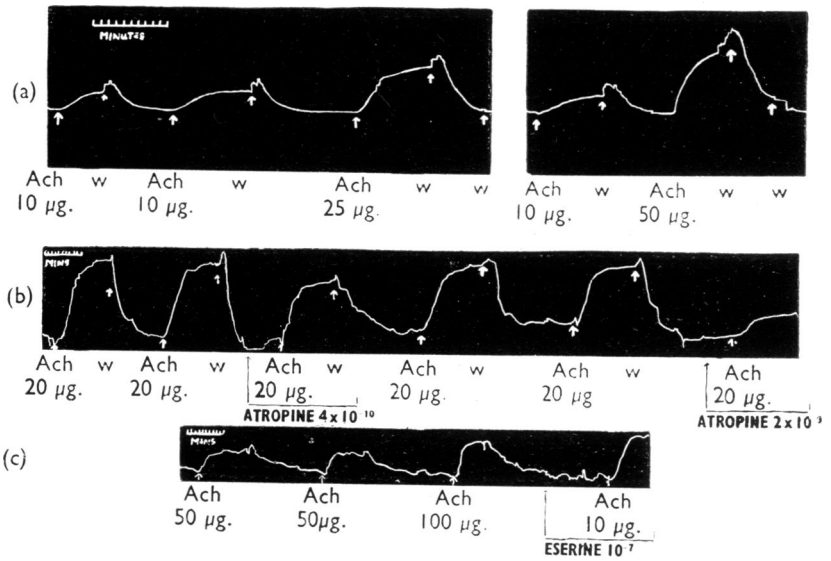

FIG. 1.—Human bronchial chains in 50 ml. modified Krebs–Henseleit solution. Intervals between injections 15–30 min. (*a*) Graded responses with acetylcholine (Ach). (*b*) Antagonism of acetylcholine by atropine. (*c*) Potentiation (> tenfold) of acetylcholine by eserine.

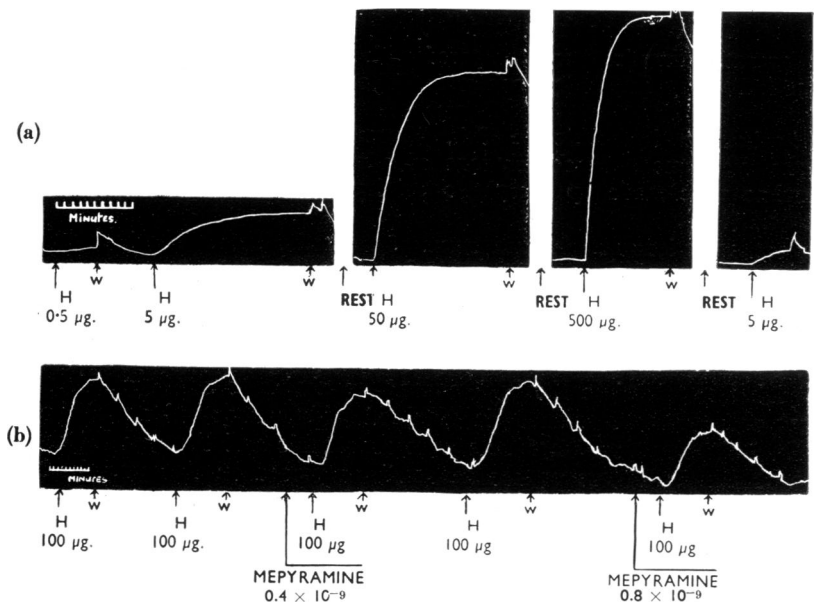

FIG. 2.—Human bronchial chain in 50 ml. bath. (*a*) Graded responses with histamine (H). (*b*) Antagonism of histamine by mepyramine.

Eserine in a concentration of 10^{-7} will potentiate the response to acetylcholine (Fig. 1*c*). Larger doses of eserine themselves cause slowly developing contractions which take a long time to reverse.

Action of histamine and mepyramine

Histamine contracts the chains when it is added to the bath. A 50 per cent contraction is obtained with concentrations of the order of 5×10^{-7}. (This figure is not directly comparable with that quoted for acetylcholine, since the maximum response obtained appears to differ for the two drugs.) Fig. 2*a* shows reversible graded responses. After several large doses of histamine, the response to a small dose is usually temporarily reduced. The active concentrations of histamine are of the same order as those which Castillo and de Beer (1947) found to contract the guinea-pig tracheal chain.

The response to histamine is reduced by mepyramine maleate in concentrations as low as 10^{-9}. This is illustrated in Fig. 2*b*.

pA_2.—pA_2 measurements (Schild, 1947) were made of this antagonism. This measurement expresses the negative logarithm of the molar concentration of an antagonist required to reduce the response to a double dose of active drug to the same height as the response to a single dose. The heights of contractions were measured from the baseline after the response had reached a steady level, generally after 10 or 15 minutes. The values were computed by interpolating graphically. A determination of pA_2 is shown in Fig. 3. The values obtained in preparations from three different lungs for periods of antagonist contact of 2, 10, and 30 minutes are shown in Table I, and compared with the values obtained by Schild (1947) and Reuse (1948) on guinea-pig ileum. The values on the human bronchi are in close agreement with those on the guinea-pig gut, except that the time required for the antagonist to reach equilibrium is longer with the bronchi.

Bronchoconstrictor action of mepyramine.—In higher concentrations, 10^{-5} and greater, mepyramine itself has a constrictor action. The response is reversible and repeatable and graded doses give graded responses, though the slope of the dose-response curve is less with mepyramine contractions than it is with contractions due to histamine (Fig. 4).

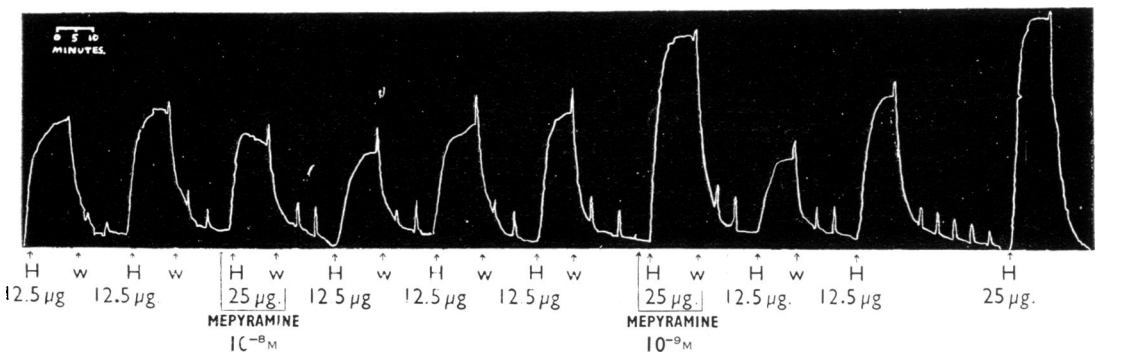

FIG. 3.—Human bronchial chain in 50 ml. bath. Determination of pA_2 for mepyramine-histamine. The value determined from this experiment was 9.27.

TABLE I

pA_2 MEPYRAMINE-HISTAMINE ON GUINEA-PIG ILEUM AND HUMAN BRONCHIAL CHAINS

	pA_2 values for different times of antagonist contact*			
	2 min.	10 min.	14 or 15 min.	30 min.
Guinea-pig terminal ileum { Schild (1947) ..	8.71		9.46	
Guinea-pig terminal ileum { Reuse (1948) ..			9.32	
Human bronchial chain ..	8.27	8.75		9.26, 9.27

* The time of antagonist contact is the time between the addition of mepyramine to the bath and the dose of histamine.

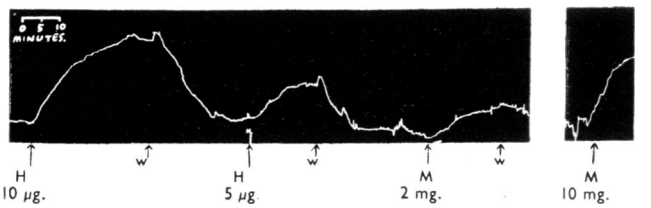

FIG. 4.—Human bronchial chain in 50 ml. bath. Bronchoconstrictor action of mepyramine (M). H = histamine.

Action of sympathomimetic compounds and aminophylline

The preparation has a good spontaneous tonus level, and sympathomimetic drugs such as *iso*propylnoradrenaline (10^{-8}), adrenaline (10^{-8}), noradrenaline (10^{-6}), and ephedrine (10^{-5}) relax the chains. Aminophylline (10^{-5}) also produces relaxation. Responses to *iso*propylnoradrenaline, adrenaline, or noradrenaline take from 10 to 25 minutes to reach a steady level, and recovery on washing out the bath takes from 20 to 40 minutes. Responses to aminophylline and ephedrine generally take longer to develop than equivalent responses to adrenaline, and the recovery from a fairly large dose of ephedrine may take as long as one hour. However, the stability of the preparation is such that it is often possible to obtain regular responses to these compounds over periods as long as 12 hours. Satisfactory graded responses are obtainable, and it is possible to compare the relative potency of bronchodilator drugs directly.

Comparative assay of bronchodilator drugs.—For this purpose we have used a four-point technique, with two doses of each drug. The order of doses within a group of four responses was randomly chosen. Fig. 5 shows a comparison of ephedrine with adrenaline. The responses were measured from the baseline to the level at which they became steady and the relative potencies computed graphically. The preparations were left at least one hour before any doses were given. Allowing for one or two preliminary doses, and doses to confirm the steadiness of the response and determine the maximum response at the end, a single comparison consisting of a group of four responses took between six and ten hours to make. Table II shows the results of comparisons of the activity of bronchodilator drugs with adrenaline obtained in different preparations at different times. Four comparisons were made with each drug, and the final result is expressed as the geometric mean of the activity ratios. As might be expected the activity ratio for the same

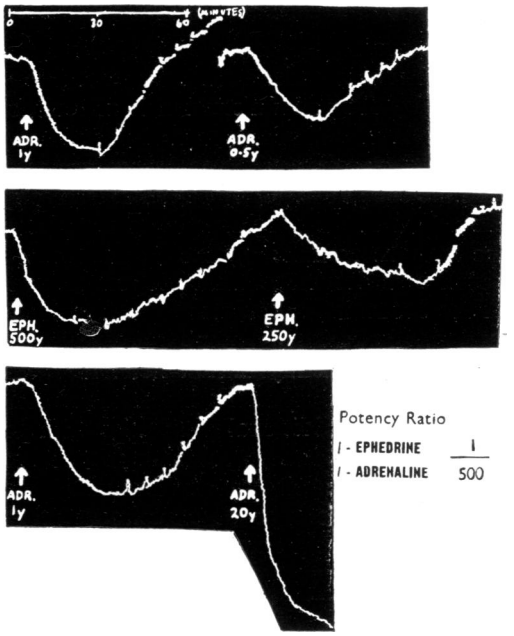

FIG. 5.—Human bronchial chain in 50 ml. bath. "Four-point" comparison of bronchodilator activity of adrenaline and ephedrine, giving a potency ratio of 500. The bath was washed out at the lowest point of each tracing.

TABLE II

ACTIVITY OF BRONCHODILATORS

	Relative potency *l*-adrenaline = 1,000	Geometric mean
*dl-iso*propylnoradrenaline	5,600 5,200 4,200 7,200	5,400
dl-noradrenaline	67 61 62 15	44
l-ephedrine	1.1 0.4 2.1 3.6	1.4
aminophylline	0.51 1.23 0.17 0.76	0.53

pair of drugs varies from experiment to experiment, but this variation is small compared with the variation between different drug pairs. The relative potencies are expressed in terms of the active base for the sympathomimetic amines and in terms of the complex salt for aminophylline.

Discussion

The evaluation of the action of drugs on the bronchi in man is usually based on vital capacity measurements. The interpretation of the changes in vital capacity caused by drug administration is difficult, since these measurements give no indication of the site of action or mode of action of the drug, which may act peripherally, centrally, or reflexly. Even if the drug is given by inhalation there is no guarantee that the effects are purely peripheral, since systemic effects are often observed. Also, the action of the drug may be to stimulate secretion of mucus, or cause oedema of the mucous membrane, thereby producing a reduction of vital capacity; it is not possible to determine what proportion of the effect observed is due to contraction of the bronchial musculature. A further complication lies in the mode of administration. Intravenous administration, which gives the most strictly comparable results, is frequently unsuitable, and when drugs are administered by inhalation their concentrations at the site of the action are not known.

There are additional difficulties with bronchodilator drugs, since in general it is necessary to induce some degree of bronchospasm with constrictor drugs and then to test the effect of the bronchodilators in relaxing this spasm. The effects recorded in this type of experiment may not indicate a general bronchodilator activity but merely demonstrate some specific antagonism. An alternative is to use emphysematous patients with some degree of pathological bronchial obstruction.

An isolated preparation of the type we have described avoids most of these difficulties and provides a direct measure of the activity of drugs on the smooth muscle of the bronchi. The chain preparation possesses the advantages that it records purely the circular component of responses and that it can be used for small bronchi. In addition it has a sufficiently high tonus level to permit direct examination of bronchodilator actions.

In general, the sensitivity of the preparation to drugs seems to resemble closely that of the guinea-pig tracheal chain. Castillo and de Beer (1947) found the guinea-pig tracheal chain to be contracted by histamine and acetylcholine and relaxed by adrenaline and aminophylline. The effective concentrations were of the same order as those required in the human bronchial preparation.

Sympathomimetic amines and aminophylline

Our results show that the sympathomimetic amines relax the resting tone of isolated human bronchial muscle and that comparisons of activity can be achieved without previously contracting the muscle with drugs. The comparative potencies of the amines we have tested show no marked differences from the results of previous workers who examined the activity of sympathomimetics on bronchial muscle that had previously been constricted by histamine, pilocarpine, mecholyl, or barium. Table III shows the results obtained on the human bronchial chain compared with values taken from the literature, and representative ranges of the therapeutic doses employed clinically in asthma.

A drug which will relax the unstimulated human bronchial chain is not only a true bronchodilator, but also can be expected to produce a bronchodilatation in the intact human subject. Comparisons of the results on human bronchial muscle with the therapeutic doses used clinically in asthma suggest that the therapeutic activities of the sympathomimetic amines tested and of aminophylline are largely dependent

TABLE III

EQUIACTIVE DOSES OF BRONCHODILATOR DRUGS

Author:	Present work	Tainter *et al.* (1934) Luduena (1942)	Konzett (1940)	Bresnick *et al.* (1949)	Therapeutic doses	
Preparation:	Human bronchial chain	Perfused guinea-pig lung	Chloralosed dog	Vital capacity (asthmatic human subjects)	Intra-venous	Sub-cutaneous
Constrictor agent:	None	Histamine, pilocarpine, barium	Pilocarpine	Histamine mecholyl		
*dl-iso*propylnor-adrenaline ..	0.19		0.1	0.5		
l-adrenaline ..	1	1	1	1	1 (dose= 0.1 mg.)	1 (dose= 0.5 mg.)
dl-noradrenaline	23	7				
l-ephedrine ..	710	335				30–120 (dose= 15–60 mg.)
aminophylline	1,900	1,250–1,870			2,500–5,000 (dose= 0.25–0.5 g.)	

on their activity in directly relaxing bronchial muscle. All these drugs, including ephedrine, actively dilate the isolated human bronchial muscle and produce constant reversible effects without tachyphylaxis. It is therefore unlikely that their activity is due to potentiation of adrenaline ; their action is probably direct.

The ratio of activity of adrenaline base and aminophylline in the bronchial chain is 1,900 ; if the ratios of their therapeutic activities were the same 0.5 g. aminophylline intravenously should produce the same effect as 0.25 mg. adrenaline intravenously. This may well correspond to their relative clinical activities and explain why aminophylline is sometimes effective in asthma when adrenaline fails (Sollmann, 1948) ; an intravenous dose of 0.5 g. aminophylline can be safely administered, whereas an intravenous dose of 0.25 mg. adrenaline cannot be given without producing severe circulatory effects.

The ratio of activity of adrenaline and ephedrine on isolated human bronchi is 710. The ratio of the customary therapeutic doses is 100 or less (subcutaneously 0.5 mg. adrenaline or 15–60 mg. ephedrine), but the true equitherapeutic ratio in asthma is almost certainly higher (Herxheimer, 1946).

Mepyramine

It is interesting to note that, although guinea-pig ileum is fifty or a hundred times more sensitive to histamine than human bronchi, the pA_2 value for mepyramine against histamine is similar in the two preparations.

The bronchoconstrictor action we have observed with higher concentrations of mepyramine is reminiscent of the contractile action of antergan and mepyramine

on the guinea-pig uterus described by Bovet and Walthert (1944). This effect may be of clinical importance, since, although, as these experiments show, the concentrations of mepyramine required to antagonize histamine added to the bath are extremely low, the concentrations which are required to reduce anaphylactic or allergic reactions are very much higher (Schild, Hawkins, Mongar, and Herxheimer, 1951).

Summary

1. A preparation for the study of drug action on human bronchi, consisting of a chain of bronchial rings, is described.

2. Acetylcholine, pilocarpine, and carbachol contract the preparation. The action of acetylcholine is antagonized by atropine and potentiated by eserine.

3. Histamine contracts the preparation, and its action is antagonized by mepyramine. pA_2 values determined for this antagonism indicate that the histamine response is reduced by mepyramine in the same concentrations as are effective on guinea-pig ileum. Higher concentrations of mepyramine have a bronchoconstrictor action.

4. Adrenaline, noradrenaline, *iso*propylnoradrenaline, and aminophylline relax the human bronchial chain. Their relative potencies have been determined.

5. The results agree closely with those found by other workers using animal preparations.

One of us (D. F. H.) is receiving a grant from the Medical Research Council.

References

Bovet, D., and Walthert, F. (1944). *Ann. pharm. franç.*, **2**, Suppl. to No. 4, 1.
Bresnick, E., Blakey, J. F., Levinson, L., and Segal, M. S. (1949). *J. clin. Invest.*, **28**, 1182.
Castillo, J. C., and de Beer, E. J. (1947). *J. Pharmacol.*, **90**, 104.
Herxheimer, H. (1946). *Brit. med. J.*, **1**, 350.
Konzett, H. (1940). *Arch. exp. Path. Pharmak.*, **197**, 27.
Krebs, H. A., and Henseleit, K. (1932). *Hoppe-Seyl. Z.*, **210**, 33.
Luduena, F. P. (1942). *J. Pharmacol.*, **75**, 316.
Macht, D. I., and Ting, G. C. (1921). *J. Pharmacol.*, **18**, 111.
Reuse, J. J. (1948). *Brit. J. Pharmacol.*, **3**, 174.
Rosa, L. (1951). *Boll. Soc. Tosco-Umbro-Emiliana Med. int.*, **1**, 26.
Schild, H. O. (1947). *Brit. J. Pharmacol.*, **2**, 189.
Schild, H. O., Hawkins, D. F., Mongar, J. L., and Herxheimer, H. (1951). *Lancet*, **2**, 376.
Sollmann, T. (1948). *Manual of Pharmacology*. Philadelphia: Saunders.
Sollmann, T., and Gilbert, A. J. (1937). *J. Pharmacol.*, **61**, 272.
Tainter, M. L., Pedden, J. R., and James, M. (1937). *J. Pharmacol.*, **51**, 371.