period of about 6 months. After this they become senescent and degenerate like the Wistar fibroblast strains. However, the A cell strains may be revived from the large stocks of cells being maintained in a liquid nitrogen depository. The cultures appear to consist of a mixture of fibroblastic and endothelioid cells. The cells have a high susceptibility to DNA viruses (pox-, herpes- and adenovirus groups) and to RNA viruses (myxo-, reo-, and picornavirus groups). They seem to be particularly susceptible to some of the rhinoviruses which have a limited host cell range.

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Use of Writhing Test for Evaluating Analgesic Activity of Narcotic Antagonists. (29963)

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The field of narcotic antagonist analgesics was opened by the discovery of Lasagna and and Beecher(1) that nalorphine (N-allylnormorphine), a narcotic antagonist, was by itself a potent analgesic in man. Although possessing the additional virtue of being non-addicting(2), nalorphine was nevertheless unsuitable for clinical use because of its high incidence of psychotomimetic and other side effects. Its analgesic activity in man was of particular interest because in animals nalorphine was an effective narcotic antagonist but showed no analgesic activity on the usual tests for potent analgesics, such as the mouse hot-plate and rat tail-flick tests. Other narcotic antagonist analgesics active in man, and apparently non-addicting, such as pentazocine and cyclazocine, were also found to be essentially inactive in animals by the usual analgesic tests(3,4). It has been suggested, in fact, that in the search for potent non-addicting analgesics, compounds showing analgesia on the hot-plate and tail-flick tests might be ruled out because they would almost certainly be addicting.

Siegmund, Cadmus and Lu(5) described a phenylquinone "writhing" test in mice which detected both narcotic analgesics and the non-narcotic antipyretic analgesics, although it was probably not entirely specific for analgesics. This test has been used in our laboratory since that time as one of our analgesic testing methods because it appeared to represent a different type of pain from that of the hot-plate or tail-flick, and a type which might more closely resemble surgical pain. Both phenylquinone and acetic acid were employed as the intraperitoneally injected writhing agent in mice and rats.

During the investigation of some narcotic antagonists about 2 years ago, we observed that one of them showed analgesic activity on the writhing test. This prompted further studies which suggested that some interesting correlations existed between the mouse and rat writhing tests and analgesic activity reported in man. In this connection, Bentley (6) has recently referred to unpublished work of R. E. Lister demonstrating that many morphine antagonists abolished the phenylquinone writhing response. We present here the results of writhing test analgesic studies on 6 narcotic antagonists: cyclazocine, levallorphan, nalorphine, naloxone, pentazocine, and (-)-3-hydroxy-N-cyclopropylmethlymorphinan*(7), designated for convenience as

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HNCM. For additional comparison, the narcotic analgesics morphine and oxymorphone were included in the study.

Materials and methods. The HNCM hydrochloride, levallorphan tartrate (Hoffmann-LaRoche), nalorphine hydrochloride (Merck), naloxone (N-allylnoroxymorphone) hydrochloride (Endo), morphine sulfate (Mallinkrodt), and oxymorphone (Numorphan) hydrochloride (Endo) were water-soluble powders. The cyclazocine† (2-cyclopropylmethyl-5,9-dimethyl-2′-hydroxy-6,7-benzomorphan) and pentazocine,† the corresponding 2-(3,3-dimethylallyl) derivative (Sterling-Winthrop Research Inst.), were dissolved in minimal amounts of dilute HCl at pH 4-5. The phenylquinone was phenyl-*p*-benzoquinone (Eastman). The mice used were CF #1 male albinos, weighing 20-24 g; the rats were CFN male albinos, weighing 100-160 g.

The writhing tests were conducted by minor modification of previous methods(5,8). The animals were injected subcutaneously with the narcotic antagonists, and 15 minutes later the phenylquinone was injected intraperitoneally. The phenylquinone was used as a 0.2 mg/ml solution in 5% aqueous ethanol. For mice the writhing dose was 0.25 ml/20 g, or 2.5 mg/kg; in rats it was 1 ml/150 g, or 1.3 mg/kg. For scoring purposes, a "writhe" in the mouse was indicated by stretching, twisting a hind leg inward, or contraction of the abdomen. In the rat there was, in addition, occasional arching of the back or rolling over. Each manifestation was counted as one writhe.

The total number of writhes for each animal was counted over a 60-minute time interval. The control value was determined from the average number of writhes produced by the phenylquinone in saline-injected side-by-side control animals. The calculation of analgesic activity was quantal, based on the percentage of animals at each dose level that showed only 50% or less of the average number of writhes of the group of saline controls. Then the ED_{50} and 95% Confidence Limits were determined by a log-probit method(9). Four or more dosage levels were used for each compound in each species, with at least 10 animals on each level.

Narcotic antagonist potency was determined by counteraction of oxymorphone narcosis in rats. The animals were injected subcutaneously with oxymorphone HCl at 1 mg/kg, which produced narcosis and loss of righting reflex within 20 minutes. At 30 minutes after the oxymorphone injection, the narcotic antagonist was injected subcutaneously on the opposite side of the animal. If the rat regained its righting reflex within 10 minutes, it was considered counteraction. Three dosage levels, with 10 rats per level, were used for each drug. The ED_{50} and 95% Confidence Limits were calculated (9) and expressed in terms of the base.

Results and discussion. In Table I are presented the results of the phenylquinone writhing test investigations in mice and rats. The analgesic potencies found in the animal studies are compared with the approximate analgesic potencies of these compounds in man, as reported in the literature(7,10,11,13) for subcutaneous or intramuscular injection. Furthermore, the analgesic potencies are compared with the narcotic antagonist potencies in rats, as determined by the above-described oxymorphone counteraction. The analgesic potencies are given with reference to morphine base as 1.0; the narcotic antagonist potencies are given with reference to nalorphine base as 1.0.

The narcotic antagonists are listed in the Table in order of increasing analgesic activity, with the 2 narcotic analgesics, morphine and oxymorphone, appended for reference. It may be seen that over a wide range of activity the relative analgesic potencies found on the writhing test, both in mice and rats, correspond roughly to the analgesic potencies reported in man. Furthermore, there is no general correlation between analgesia and narcotic antagonist potency. Thus, naloxone had essentially no analgesic activity in mice, rats, and man, but it was the most potent narcotic antagonist. On the other hand, 2 of the more potent narcotic antagonists, cyclazocine and HNCM, were extremely potent analgesics in both animals and man, being about 20 to 30 times as active as morphine base (or up to about 40 times as active as morphine sulfate).

† Supplied through the kindness of Dr. L. S. Harris, Sterling-Winthrop Research Institute.

TABLE I. Comparison of Writhing Test Analgesia with Clinical Analgesic Potency and Narcotic Antagonist Activity.

Compound	50% writhing prevention ED_{50} (95% C.L.) Mouse, mg/kg (base)		Rat, mg/kg (base)		Analgesic potency (as the base) Mouse	Rat	Man	Narcotic antagonist activity—rat ED_{50} (95% C.L.), mg/kg (base)		Potency
Naloxone	>82		>41		<.007	<.005	0*	.045	(.027-.071)	19
Levallorphan	26	(17-42)	1.7	(.79-3.8)	.022	.11	0*	.24	(.14-.41)	3.4
Pentazocine	3.8	(2.5-5.9)	.95	(.53-1.7)	.16	.21	.2†	16	(11-24)	.051
Nalorphine	.48	(.29-.82)	.20	(.12-.31)	1.2	1.0	.9‡	.81	(.52-1.3)	1.0
Cyclazocine	.028	(.013-.061)	.012	(.005-.025)	21	17	30‡	.45	(.30-.68)	1.8
HNCM	.019	(.010-.036)	.018	(.011-.030)	31	11	30§	.40	(.27-.61)	2.0
Morphine	.59	(.48-.73)	.20	(.14-.29)	1.0	1.0	1.0			.0
Oxymorphone	.041	(.024-.069)	.018	(.012-.027)	14	11	8.5‡			.0

* Foldes(13). † Keats and Telford(11). ‡ Lasagna(10). § Gates and Montzka(7).

The only significant discrepancy appeared in the case of levallorphan, in which very little analgesic activity was found in mice, whereas in rats it was about one-ninth as active as morphine, or about one-half as active as pentazocine. In man levallorphan is considered to have little or no analgesic activity. The comparative narcotic antagonist potencies in rats agree approximately with those reported in the literature for different methods (7,14,15), except for slightly lower potencies for cyclazocine and HNCM in the present experiments.

It is of interest that in the writhing tests oxymorphone was found to be approximately 14 times as potent as morphine in the mouse and 11 times as potent in the rat, which is in rather good agreement with the analgesic potencies of 15 (mouse) and 16 (rat) found in this laboratory in the Eddy hot-plate test. Cyclazocine and HNCM were slightly more potent than oxymorphone on these mouse and rat writhing tests for analgesia, as well as in man, apparently(7,12,16). In contrast to oxymorphone, however, cyclazocine and HNCM have little or no analgesic activity on the mouse hot-plate or rat tail-flick tests (4,7).

The 6 narcotic antagonists investigated in this study represent compounds derived from 4 different parent narcotic analgesics: morphine, oxymorphone, levorphanol, and phenazocine. Despite the wide span of analgesic activity covered by the group of antagonists, ranging from essentially no activity in naloxone to a potency of about 20 times morphine in cyclazocine and HNCM, the results of the mouse and rat writhing tests appear to agree approximately with the analgesic potencies observed in man. Inasmuch as the evaluation of narcotic antagonist analgesics in animals has proved so difficult in the past, further investigation of the writhing test, with additional antagonists, is highly desirable to determine the reliability with which it mirrors analgesic potency of narcotic antagonists in man.

Summary. In a comparison of 6 narcotic antagonists, the analgesic potencies found on the phenylquinone writhing test, in both mice and rats, roughly paralleled the analgesic potencies reported in man. The analgesic po-

tencies did not correlate with the narcotic antagonist potencies. In view of the fact that the narcotic antagonist analgesics are inactive on the usual analgesic tests in animals, it appears that the writhing test may be a valuable aid in attempting to predict the analgesic activity of narcotic antagonists in man.

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Experimental Caries Induced in Animals by Streptococci of Human Origin.* (29964)

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Traditionally, lactobacilli have been considered the principal cause of caries, but recent findings have questioned this concept. Orland(1) succeeded in producing experimental caries by infecting gnotobiotic rats with a strain of streptococcus. More recently, Fitzgerald *et al*(2) confirmed these findings, and Fitzgerald and Keyes(3) established the cariogenicity of another strain of streptococcus in hamsters. These 2 strains of streptococci were shown to be host-specific with regard to cariogenicity: the hamster strain (designated HS_1) produced caries only in the hamster and not in the rat, and the rat strain (designated FA_1) produced caries in the rat and not in the hamster. Fitzgerald and Keyes also demonstrated that experimental dental caries in animals is an infectious and contagious pathological state which could be transmitted from mother to young.

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Studies of cultures from human caries have brought out evidence for the presence of streptococci similar to the HS_1 and FA_1 strains. Preliminary findings indicate that the human streptococci similar to the HS_1 strain may be causally associated with human caries. Studies related to human streptococci similar to FA_1 strains are being investigated.

Materials and methods. Collection of specimens from human carious lesions. A sterile spoon excavator was used to remove carious material from the depths of lesions in both enamel and dentine from human teeth *in situ*. This material was inoculated in Todd-Hewitt broth, supplemented with 0.5% lactalbumin hydrolysate and incubated at 37°C for 18-24 hours.

Production of antiserum. Rabbits were immunized with a vaccine prepared from heat-killed streptococcal cultures (60°C for 1 hr). The bacterial suspensions were washed twice in saline and adjusted to the density of the