differences in growth potential exhibited in the unconditioned hamster can be evaluated as differential evidence of malignancy in general. However, the present data can be interpreted as representing distinct biological differences among those cell lines derived from normal and neoplastic tissues which can be maintained in tissue culture.

Summary. Quantitated inocula of 14 tissue culture cell lines which are remarkably similar *in vitro* were implanted into cheek pouches of unconditioned Syrian hamsters. All cell lines grew in the cheek pouch when 1 x 10^6 cells were implanted, but only those cell lines derived from neoplastic tissue produced tumors when inocula contained 1 x 10^4 cells. Other experiments indicated that this difference in the growth potential of cell lines deriving from neoplastic tissue also may be delineated in hamsters conditioned with cortisone acetate by implantation with 1 x 10^3 or fewer cells.

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Antagonism of Insulin-Induced Gastrointestinal Hypermotility in the Rat.* (23045)

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Antispasmodic potencies of many drugs have been expressed in terms of relative degree of antipropulsive action shown in the Macht and Barba-Gose charcoal meal test(1). The results of this test are informative, but effects of drugs on normal propulsive motility may not reflect useful effects expected in treatment of gastrointestinal malfunctions associated with smooth muscle spasm and hypermotility. Some clinically useful agents, such as adiphenine hydrochloride (Trasentine) and dicyclomine hydrochloride (Bentyl), which exert their smooth muscle inhibiting effect in "musculotropic" or "papaverine-like" fashion, are without significant inhibitory effect in the charcoal meal test except in doses which produce pronounced central nervous system effects. Atropine sulfate and methantheline bromide (Banthine) require subcutaneous doses of 2 mg/kg and 15 mg/kg respectively to produce a 50% decrease in normal propulsion. Such high doses of these agents might be expected to be responsible for extraneous effects which could be antagonistic or synergistic with peripheral antipropulsive action of the compound. The charcoal meal test alone fails to indicate ex-

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TABLE I. Charcoal Meal Test. Effect of drugs on normal propulsive motility of stomach and small intestine of rat.

No. of rats	Agent	Dose, mg/kg	Route of admin.	Avg length intestine traversed (% ± S.D.)† 20 min.	40 min.
25	Saline		S.C.		61.5 ± 12.0
20	"		Oral	51.4 ± 9.5	
5	Atropine SO_4	1	S.C.		50.8 ± 10.3
10	*Idem*	2	"		34.3 ± 6.1
10	"	4	"		26.7 ± 5.4
10	"	8	"		35.7 ± 5.1
5	"	16	"		36.1 ± 5.3
5	"	32	"		33.0 ± 13.6
5	"	8	"	27.6 ± 11.0	
5	"	2	Oral	30.1 ± 3.5	
5	"	8	"	36.9 ± 9.8	
5	"	16	"	37.1 ± 3.5	
5	Methantheline br.	2	S.C.		56.1 ± 8.2
5	*Idem*	8	"		52.2 ± 6.9
15	"	16	"		39.0 ± 3.9
10	"	32	"		13.1 ± 5.0
5	"	100	Oral	24.2 ± 6.1	
10	Scopolamine methylbr.	1	S.C.		31.0 ± 4.3
10	*Idem*	2	"		28.5 ± 6.5
5	Papaverine HCl	100	"		54.9 ± 10.6
10	*Idem*	200*	"		21.7 ± 10.7
10	"	400*	"		6.6 ± 6.7
5	"	200*	Oral	54.5 ± 9.2	
5	"	400*	"	32.0 ± 6.3	
10	Dicyclomine HCl	125	S.C.		38.8 ± 5.5
5	*Idem*	250*	"		21.3 ± 5.4
10	Adiphenine HCl	200*	"		53.2 ± 13.2
10	Hexamethonium br.	2	"		53.6 ± 11.2
10	*Idem*	4	"		41.0 ± 9.5
10	"	8	"		31.9 ± 8.2
5	"	16	"		37.1 ± 10.6

* Symptoms of depression. † Distance to farthest point of charcoal.

tent to which acetylcholine blocking capacity, ganglionic blocking capacity, or direct smooth muscle inhibiting capacity of a compound contributes to its total antipropulsive action. Additional use of a similar *in vivo* test, which would allow comparison of effect of the drug on both normal and hyperactive states of intestinal smooth muscle of the same species, should help answer this question.

Methods. Adult rats of the Mead Johnson colony (McCollum strain) and of either sex weighing between 200 and 400 g were caged without food and water for 24 hours prior to experiment. The test drug was injected subcutaneously and followed immediately by subcutaneous dose of 40 units/kg of insulin (Squibb), each in 2 ml/kg volume, and each injected at a different site. Thirty minutes later 5 ml/kg of the charcoal mixture (6% charcoal and 1% methocel 400 in water) was administered orally. Forty minutes after administration of the charcoal mix the animals were sacrificed with diethyl ether and stomach and small intestine removed. The distance from the stomach to the nearest area which contained the first one cm, or more, of charcoal and also the distance from stomach to farthest point containing one cm or more of charcoal, were expressed as % of total length of small intestine. The insulin-treated rats were somewhat physically depressed but in experiments on 800 rats, only one convulsion was seen during the 40-minute waiting period. Distribution of the charcoal mixture in insulin-treated rats is different from that in the Macht and Barba-Gose rats and to our

knowledge the distribution in insulin-treated animals has never been described. Forty to 50 minutes after oral administration of 5 ml/kg of charcoal mixture, according to the procedure of Macht and Barba-Gose, this mixture is rather uniformly distributed in the proximal 60% of small intestine and the stomach appears to be rather full. In the insulin-treated rats the stomach is small and firm with relatively little retained material. No charcoal mix is found in the proximal 34% of the intestine (average of 60 rats), and the farthest point containing one cm or more of the charcoal is usually 70 to 80% of length of small intestine. The major, and most consistent, result of insulin administration was the "cleaning out" of stomach and proximal 34% of small intestine. This result is apparently in agreement with the conception that the predominant effect of insulin is exerted, via autonomic nerves, from hypoglycemic stimulation of vagal centers in the central nervous system and that the stomach and more proximal portions of the gastrointestinal tract are more richly supplied with vagal fibers(2). It was found that there was an approximately linear relationship between the log-dose of antipropulsive agents and the average inhibition of insulin effect as determined by measuring the distances from stomach to beginning of charcoal mix. Although insulin caused an increase in the distance traversed by the farthest portion of the charcoal mix, this increase was not well defined, and it soon became apparent that there may be no relationship between the effect of a drug on inhibiting the "cleaning out" action and reducing the distance of the farthest point of travel. This latter measurement was therefore not used in determining ability of drugs to antagonize the hyperpropulsive effects of insulin. The procedure employed in the charcoal meal test was the same except that rats received no insulin, and the farthest point at which one cm or more of the charcoal mix was found was used to calculate the % intestine traversed. The difference in this distance between treated rats and control rats was expressed as % inhibition. Effectiveness of the following antispasmodic agents was compared in the 2 tests: atropine sulfate, papaverine hydrochloride, methantheline bromide (Banthine), adiphenine hydrochloride (Trasentine), dicyclomine hydrochloride (Bentyl), scopolamine methylbromide (Pamine), and hexamethonium bromide. All doses were based on weight of the salt.

TABLE II. Effect of Drugs on Gastrointestinal Hyperpropulsion Induced by Subcut. Admin. of Insulin in the Rat.

No. of rats	Agent	Dose, mg/kg	Route of admin.	Avg distance,* stomach to charc'l mix at 40 min., % ± S.D.
60	None			34.4 ± 8.2
10	Atropine SO_4	.1	S.C.	21.2 ±11.0
5	*Idem*	.25	"	18.9 ± 7.2
10	"	.5	"	6.6 ± 4.4
10	"	1	Oral	27.5 ± 5.1
20	"	2	"	15.3 ±16.4
10	"	4	"	4.7 ± 4.8
10	Methantheline	.5	S.C.	19.4 ± 7.2
10	br.	2	"	7.9 ± 6.5
5	*Idem*	4	"	5.5 ± 4.3
10	"	40	Oral	20.9 ±10.3
15	"	80	"	7.5 ± 5.0
10	Scopolamine	.0125	S.C.	24.7 ± 5.3
10	methylbr.	.05	"	15.2 ± 4.2
10	*Idem*	.15	"	0
10	"	4	Oral	20.0 ±13.7
10	"	8	"	15.2 ± 4.6
10	"	12.5	"	2.6 ± 4.2
10	Papaverine HCl	12.5	S.C.	18.3 ± 6.5
10	*Idem*	25	"	14.8 ± 6.7
10	"	50	"	2.8 ± 0.3
10	†Adiphenine HCl	50	"	19.4 ±14.1
5	*Idem*	100	"	18.1 ± 2.6
10	Dicyclomine	5	"	22.0 ± 4.6
10	HCl	10	"	20.0 ± 9.1
10	*Idem*	20	"	14.8 ± 6.8
10	Hexametho-	1	"	24.4 ± 8.6
10	nium br.	2	"	16.2 ± 5.4
5	*Idem*	4	"	0

* Distance to nearest point of charcoal.
† Commercially available solution containing glucose.

Results. Table I and Table II list the results obtained in the charcoal meal test and insulin test. Since some investigators have administered the compounds orally and utilized a 20-minute waiting period in their charcoal meal tests, some data obtained in this manner are included for purposes of comparison.

The relative potency of each drug in both

TABLE III. Comparison of Potency of Several Antipropulsive Agents as Measured by Different Methods in the Intact Rat.

Drug	Charcoal meal test S.C. ED_{50}* (mg/kg)	Insulin antipropulsion test	Ratio, char. meal ED_{50}/insulin ED_{50}
Atropine SO_4	2.4	.146	16.4
Methantheline br.	16.5	.66	25.0
Scopolamine methylbr.	1.05	.028	37.5
Papaverine HCl	165	15.5	10.6
Dicyclomine HCl	157	13.0	12.1
Hexamethonium br.	8†	1.82	4.61

* ED_{50} was determined from log dose-response relationships and represents approximate dose required to produce an avg of 50% inhibition of propulsion as described in methods.

† Produced an avg of 48% inhibition in group of 10 rats.

the charcoal meal test and insulin antipropulsion test is shown in Table III. To eliminate variability of absorption from the gut, all comparisons were made from data obtained with subcutaneous injections of antipropulsive agents.

The slopes of the log dose-response lines for atropine, methantheline and scopolamine methylbromide in the insulin antipropulsive test were very similar, while those of dicyclomine and papaverine were much flatter but similar to each other. With the exception of scopolamine methylbromide and hexamethonium bromide, antipropulsive potencies, relative to atropine, were quite similar in either test. Papaverine and dicyclomine, when compared to atropine, were more effective in the charcoal meal test and relatively less effective in the insulin test, whereas the reverse was true for methantheline and especially scopolamine methylbromide. The results indicate that the more a compound relies upon its papaverine-like effects to inhibit normal propulsion, the nearer to unity will be the ratio between the ED_{50} in the two tests.

The data summarized in Table IV give some indication of the specificity and limitations of the insulin antipropulsion test. The results are in agreement with the general concepts of the actions of these drugs except in the case of dibenamine and phentolamine. Despite the fact that the known peripheral effects of these agents would not be expected to interfere with the insulin-induced hypermotility, it is seen that they are capable of producing pronounced inhibition in doses incapable of affecting normal propulsion. The possibilities of parasympathetic ganglionic blocking action or a central blocking action are now being investigated.

Discussion. Methantheline is known to possess approximately ½ the ganglionic blocking capacity of hexamethonium(3), and since it was only half as potent as hexamethonium in the charcoal meal test, its ganglionic action was probably of importance. In the insulin antipropulsion test, on the other hand, methantheline was 3 times as potent as hexamethonium, making available only ⅙ the necessary ganglionic blocking activity required to reduce insulin-induced propulsion. The fact that hexamethonium was so efficient in abolishing hyperpropulsive action of insulin is consistent with the idea that extrinsic nerve impulses affect the increased motility of the gut. The inhibitory effect exerted by procaine and lidocaine is probably due to ability of these agents to interfere with ganglionic transmission. The comparative potency of various ganglionic blocking agents will be the subject of a subsequent report.

TABLE IV. Effect of Subcutaneous Injections of Various Agents on Normal Propulsion and Insulin-Induced Hyperpropulsion in the Intact Rat.

No. of rats	Agent	Dose, mg/kg	% inhibition: Charcoal meal test	% inhibition: Insulin antipropulsion test
10	Hexamethonium	1		29.1
10	br.	2	12.9	53.0
5	*Idem*	4	33.4	100.0
10	"	8	48.2	
5	"	16	40.0	
5	Pyrilamine maleate (Neoantergan)	2	0	0
10	Phenoxybenzamine	.1		39.1
5	(Dibenzyline)	1	0	100.0
5	Phenoxybenzamine	10	41.7	
10	Phentolamine HCl	.2		28.8
10	(Regitine)	2	0	72.8
10	*Idem*	10	16.6	
10	Procaine HCl	6		31.3
5	*Idem*	40	23.0	
10	Lidocaine HCl	2		46.6
5	(Xylocaine)	10	4.0	

Atropine appears to be definitely limited in its ability to inhibit normal propulsion as shown by the fact that a subcutaneous dose of 2 mg/kg is apparently as effective as 16 times that dose. The results suggest that higher doses of atropine introduce a factor which opposes peripheral inhibition of propulsion. An exaggeration of this phenomenon could be expected in the case of new synthetic agents whose actions may be less specific than those of atropine and other agents included in this report.

The insulin antipropulsion test has several advantages over the charcoal meal test for comparing activity of new antipropulsive agents. Besides economy and convenience afforded by the fact that smaller doses of drugs are required, one is much less likely to encounter unwanted side effects which may prevent a true evaluation of the antipropulsive potency. Because papaverine-like agents, in otherwise asymptomatic doses, are capable of producing pronounced inhibition of the propulsive effect of insulin, one is much less likely to overlook an important agent of this type. Finally, a comparison of the effect of the compound in both tests may be very informative.

Summary. A new intestinal antipropulsive test in intact insulin-treated rat is described. The insulin test provides certain advantages over the commonly-used charcoal meal test for screening of synthetic antispasmodic or antipropulsive agents. The use of both tests permits determination of the effect of the agent on both normal and hyperactive states of intestinal muscle and provides information relative to the mechanism of action of the agent. The specificity and some limitations of the test are discussed.

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Glomerular Filtration Rate and Renal Plasma Flow in Cholera and Acute Gastroenteritis. (23046)

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Cholera is characterized by acute purging and vomiting leading to marked dehydration of body and acute depletion of plasma volume. The renal plasma flow is likely to be interfered with and either transitory or permanent damage to kidney might be possible. Death is mostly due to development of anuria which indicates failure of function of the kidney. Hence assessment of kidney functions is of importance to predict prognosis, to learn progress of the disease and to suggest treatment. Anuria is invariably present but not so in conditions of acute gastroenteritis, though clinical features resemble each other closely. Whether the hemodynamic factors in the formation of urine are altered in the above 2 conditions, either due to effect of specific toxins or to depletion of plasma volume, remains to be elucidated. In the present investigation glomerular filtration rate and renal plasma flow were determined in patients suffering from cholera and acute gastroenteritis. The same studies were also carried out in normal persons for comparison.

Methods. Selection of cases. All patients suffering from cholera and gastroenteritis admitted into the Nilratan Sircar Medical College Hospitals, with clinical features of acute purging, vomiting and showing signs of dehydration were selected. As soon as patients were admitted, their dehydration was corrected by transfusion of hypertonic saline followed by normal saline with sodium bicarbonate or hypertonic glucose(25%) with calcium gluconate and ascorbic acid. Patients whose stool on culture did not show *vibrio*