ture of incomplete and infectious virus remains to be studied.

Summary. HeLa cells infected with herpes simplex virus produced more organic acids than uninfected control cultures when glucose was the principal substrate. The organic acids which accumulated in growth fluids were separated by celite chromatography and further identified by ascending paper chromatography. Infected cells grown with cortisone formed as much lactic acid as infected cells grown in absence of cortisone, but in addition, the former cells produced large amounts of acetate, pyruvate and another compound tentatively identified as succinate. Glucose utilization was stimulated by virus and cortisone in the HeLa cell system resulting in accumulation of Krebs cycle intermediates. Concomitant with marked acid production definite cytopathogenicity and marked increases in virus titers occurred in the virus-infected cultures. It may be concluded that the herpes virus-cortisone association alters carbohydrate metabolism of HeLa cells in a striking pattern.

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Effect of 1-(3-Hydroxyphenyl) 2-Methylaminoethanol (Phenylephrine) and 1-(3-Hydroxyphenyl) 2-Aminoethanol (Norphenylephrine) on Intestinal Motility. (24778)

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Norphenylephrine (differing from phenylephrine in that it has a hydrogen atom instead of a methyl group on the nitrogen atom) has not been fully investigated with regard to its effects on smooth muscle of the type which is inhibited by epinephrine. Kuschinsky and Oberdisse(1) reported that norphenylephrine has an inhibitory action on rat uterus with a potency 1/10 that of epinephrine, and Boyd (2) reported that norphenylephrine has an excitatory action on rabbit uterus. According to Lands(3) norphenylephrine is inactive as a bronchodilator. As an inhibitor of the peristaltic reflex in the guinea pig ileum phenylephrine and norphenylephrine were found to be 1/5000 and 1/6000 as potent, respectively, as epinephrine, while they were 1/10 and 1/50 as potent as epinephrine in causing inhibition of the "pendular movements" in the isolated rabbit ileum(4). In the present study a comparison is made between the rabbit intestine-inhibiting actions of phenylephrine and norphenylephrine; and differences in potency of these compounds are found to be much less

than those reported by McDougal and West (4).

Methods. Studies of relative potency of l-phenylephrine, dl-phenylephrine, and dl-norphenylephrine were performed on isolated strips of rabbit jejunum. The rabbit was killed by injection of air intravenously. Strips of jejunum were removed and rinsed 4 times in Locke's solution which was oxygenated and maintained at a temperature of 37°C. Segments 2-3 cm long were removed and suspended in a muscle bath containing 49 ml of oxygenated Locke's solution held at a temperature of 37°C ± 0.5°. The strips were attached to writing levers which recorded the muscle excursions on a kymograph drum. Conditions were the same for each test solution. Approximately 10-15 minutes were allowed for the strip to show relatively constant tonus and motility. A test solution of one ml was pipetted into the muscle bath and the effects recorded. Immediately following this the bath was drained and 49 ml of fresh Locke's solution was replaced. The next test solution was not introduced until contractions of the isolated muscle had returned to normal. All intestinal strips were studied on the same day that they were removed from the animal. The effect on isolated rabbit intestine of a test dilution of l-epinephrine, usually one part in 20 million or one part in 10 million, was determined. Following this a series of dilutions of l-phenylephrine, dl-phenylephrine, and dl-norphenylephrine were tested to determine which would duplicate the inhibitory effect of the test dilution of l-epinephrine. Epinephrine was introduced either before or after each test dilution in all cases. Similar technics were used in comparing the potency of dl-norphenylephrine and dl-phenylephrine. In the assay comparing dl-norphenylephrine and dl-phenylephrine identical concentrations of stock solutions (1.0 mg/ml) were used. Test solutions were of the range of 0.0002 mg/ml to 0.0012 mg/ml for both drugs. All dilutions described above include correction for a 50 ml dilution factor since the volume of the bath was 49 ml. The dl-form of phenylephrine was used rather than the l-form, since the l-form of norphenylephrine could not be obtained. None of the stock solutions of the drugs were kept for more than one day.

Results. Both phenylephrine and norphenylephrine when given in adequate amounts had effects on the isolated rabbit intestine similar to the effects of l-epinephrine. There occurred a decrease in tonus and reduction in amplitude of or cessation of rhythmic contractions with hyperactivity sometimes following the inhibition.

TABLE I. Comparison of the Relative Potency of L-Phenylephrine, DL-Phenylephrine and DL-Norphenylephrine with That of L-Epinephrine. Number of times the dose of l-phenylephrine, dl-phenylephrine and dl-norphenylephrine must be increased beyond that of a test dose of l-epinephrine in order to duplicate degree of inhibition of intestinal motility produced by l-epinephrine. Reciprocal of any figure in the table indicates potency of that compound as compared with that of l-epinephrine.

Rabbit	Segment No.	l-epinephrine	l-phenylephrine	dl-phenylephrine	dl-norphenylephrine
A	1	1	5–6, 4–5		
B	1	1	4–5, 5–6		
C	1	1		8–12	
	2			10–14	
D	1	1			22–24
	2	1			20–24
E	1	1			8–10, 10–12
	2	1			8–10, 12–14
F	1	1			12–16
	2	1			12–14
G	1	1			10–14
	2	1			8–14
H	1	1			20–24
	2	1			18–24
Range		1	4–6	8–14	8–24

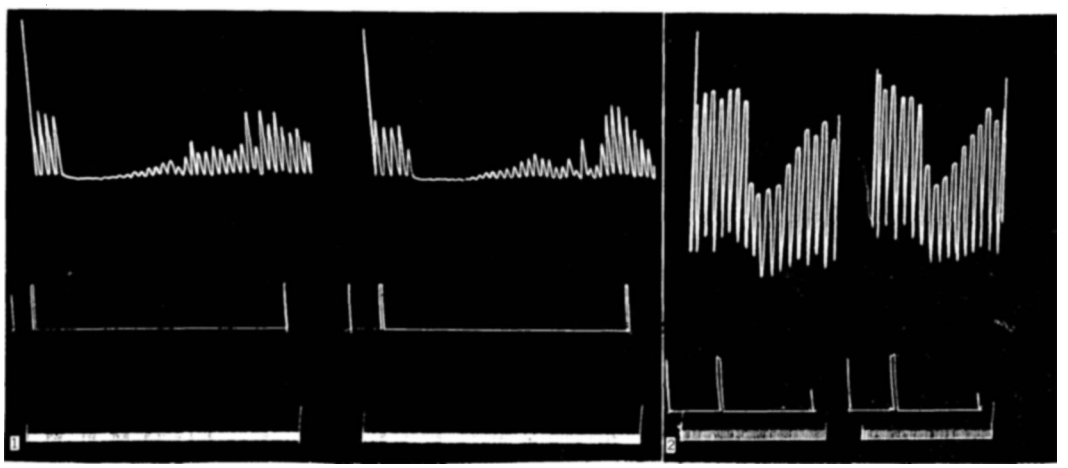

FIG. 1. Tracings of effect of 0.0005 mg/ml of dl-norphenylephrine and 0.00005 mg/ml of l-epinephrine, on motility of isolated jejunal strips. From above downward tracings in each figure show motility of intestinal segment, point of application of compound and time in one sec. intervals.

FIG. 2. Tracings of effect of 0.0002 mg/ml of dl-phenylephrine and 0.0004 mg/ml of dl-norphenylephrine respectively.

I. *Comparison of actions of l-phenylephrine, dl-phenylephrine and dl-norphenylephrine with action of l-epinephrine.* In 2 rabbits the action of l-phenylephrine was compared with l-epinephrine. In 4 assays it was found to be 1 4 to 1 6 as potent. This is within the range previously reported by Aumann and Youmans(5). In tests of the effects of dl-phenylephrine on 2 segments from one rabbit it was found to be 1/8 to 1/14 as potent as l-epinephrine. This is in accord with the well known fact that the dextro forms of phenylethylamine derivatives are relatively impotent, so that if the racemic form is used the potency of the solution is approximately ½ that of the potency of the levo form. The actions of dl-norphenylephrine were compared with those of l-epinephrine in duplicate tests on segments from 5 rabbits. In Fig. 1 a typical record obtained during this study is illustrated. The figure shows what was considered to be equal effects. The results are shown in Table I (rabbits D through H inclusive). It can be seen that dl-norphenylephrine is from 1/8 to 1/24 as potent as l-epinephrine.

II. *Direct comparison of action of dl-norphenylephrine with that of dl-phenylephrine.* The actions of dl-norphenylephrine were compared with those of dl-phenylephrine in a total of 14 assays on 2 segments from each of 4 rabbits. A typical record obtained during this study is illustrated in Fig. 2. The results of these assays are presented in Table II. It was found that a dose of dl-norphenylephrine must be increased 2 to 2.6 times beyond that of a test dose of dl-phenylephrine in order to duplicate degree of inhibition of intestinal motility produced by dl-phenylephrine. Thus, demethylation of the compound dl-phenylephrine reduces its potency as an inhibitor of intestinal motility to about 1/2 to 2/5 that of its original potency. Relative potency of the 2 compounds apparently can be established somewhat more closely by comparing

TABLE II. Comparison of Relative Potency of DL-Norphenylephrine with That of DL-Phenylephrine. Number of times the dose of dl-norphenylephrine must be increased beyond that of a test dose of dl-phenylephrine in order to duplicate degree of inhibition of intestinal motility produced by dl-phenylephrine.

Compounds	Rabbit AA		Rabbit BB		Rabbit CC		Rabbit DD		Range
	Seg. 1	Seg. 2	Seg. 1	Seg. 2	Seg. 1	Seg. 2	Seg. 1	Seg. 2	
dl-phenylephrine	1	1	1	1	1	1	1	1	1
dl-norphenylephrine	2.3*	2.2*	2.2–2.5†	2–2.5†		2–2.6‡	2.4§	2–2.4§	2–2.6

Data based on: * 1 assay. † 3 assays. ‡ 4 assays. § 2 assays.

them with each other than by comparing them with epinephrine as a common standard. The results of both methods indicate that the per cent loss of intestine-inhibitory potency of phenylephrine which results from demethylation is of the same order as that observed when the methyl group is removed from epinephrine(5). Thus removal of the methyl group has the same effect on intestine-inhibitory potency whether the para OH group is present as in the case of epinephrine or absent as in the case of phenylephrine, and the amount of loss of potency is slight relative to the effect of removing any of the hydroxyl groups.

Summary. (1) The effects of dl-phenylephrine and dl-norphenylephrine on motility of isolated strips of rabbit intestine were studied. Comparisons of their actions were made with the action of l-epinephrine and with each other to obtain relative potency of these phenylethylamine derivatives as inhibitors of intestinal smooth muscle. (2) The potency of dl-norphenylephrine as an inhiibtor of isolated rabbit intestine is approximately one-half that of dl-phenylephrine.

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Immune Hemolysis: Determination of Values for Optimal and Maximal Sensitization. Comparison in Several Hemolytic Systems. (24779)

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The role of Complement (C′) in the mechanism of immune hemolysis has received a great deal of study. Yet, relatively little attention has been devoted to the other participant of immune hemolysis, namely the sensitized cell. Recent studies by Talmage and co-workers(1) have afforded some insight as to the role played by the sensitizing antibody in immune hemolysis. We observed(2) striking differences in amount of C′ required to produce comparable lysis in various hemolytic systems, sensitizing the cells with optimal amounts of hemolysins and using the identical C′. Further, the number of hemolytic units required for optimal sensitization also differed from one system to another. These findings were characteristic of the particular system and were apparently reflections of differences in immunological properties of the sensitized cell. It therefore seemed feasible to use such information in characterization of immune hemolytic systems. The practical aspects of the concept of optimal sensitization in immune hemolysis were presented by Osler, Strauss and Mayer(3). Determination of amount of hemolysin required for optimal sensitization was used for selecting suitable hemolysins and determining amount of hemolysin which should be used for sensitization. By refining the original method it was possible to obtain a more exact determination of this value. It was found that amount of antibody required for optimal sensitization did not necessarily represent maximal amount of antibody capable of combining with the cells. Therefore, it was also possible to obtain a value for maximal sensitization, based upon hemolytic absorptive capacity of the cell. In the present communication we determined values for optimal and maximal sensitization in each of 4 hemolytic systems and have shown the relationship of these values to C′ activity. *Materials and methods* were essentially the same as previously described(2). Certain modifications and additional procedures are given below: *Complement.* Lyophilized guinea pig C′ (Sharpe and Dohme) was reconstituted and absorbed once with washed and packed hu-