one in each circuit, for blood to pass from one system to the other.

Summary. Apparatus and technic for cross-dialysis is described. It is believed that the procedure is relatively uncomplicated, safe and requires few laboratory checks during operation. Only further extensive studies will determine whether or not it has any value as a clinical tool.

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Received February 19, 1953. P.S.E.B.M., 1953, v82.

Conjugation of N-Allylnormorphine by Liver Slices.* (20165)

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The respiratory depression caused by an overdosage of morphine can be abolished by small amounts of N-allylnormorphine† (1,2,3). N-Allylnormorphine and morphine differ only in the alkyl group attached to the nitrogen; morphine has a methyl group at this position, whereas N-allylnormorphine has an allyl group attached to the nitrogen. Previous reports(4) have stated that the phenolic hydroxyl group in morphine is the site primarily responsible for the effect on respiration, but now it is evident that changes on the nitrogen atom are important in this respect.

In man(5) and dogs(6) morphine has been reported excreted in urine in both a free and combined state. A study of the action of liver slices on N-allylnormorphine was undertaken to determine whether it was conjugated in the liver like morphine(7) and whether there might be a significant difference in the rate of conjugation to account for the blocking ratio of one molecule of N-allylnormorphine to sixty-seven molecules of morphine (8).

Methods and results. The procedure of Snell and Snell(9) as used by Bernheim(7) was used for the estimation of morphine. This method probably involves the reactivity of the phenolic and alcoholic hydroxyl groups of morphine, for codeine and dihydromorphinone, in which these groups are altered, do not give the same intensity of color as morphine. N-allylnormorphine is identical to morphine except for the replacement of the methyl group on the nitrogen by an allyl group and would be expected to give the same color intensity with the Snell and Snell reagent. That this is so is shown in Fig. 1. The color developed with the silicomolybdic acid reagent is the same for both morphine and N-allylnormorphine in the concentrations used.

In order to study the rate of conjugation of morphine and N-allylnormorphine, livers from dogs anesthetized with sodium pentobarbital were sliced in the usual way. About 300 mg of tissue (wet weight) was placed in 4 ml of Krebs-Ringer bicarbonate solution with either 1.0 mg of morphine or N-allylnormorphine. The slices were incubated at 38°C with a gas phase of 5% carbon dioxide and 95% oxygen in a Dubnoff shaker. Suitable

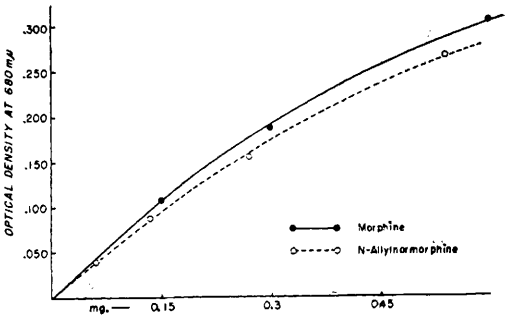

FIG. 1. A comparison of the optical density with the silicomolybdic acid reagent between morphine and N-allylnormorphine.

* This investigation was supported in part by a research grant from the National Institute of Health, Public Health Service.

† N-Allylnormorphine was obtained through the courtesy of Merck & Co., Rahway, N. J.

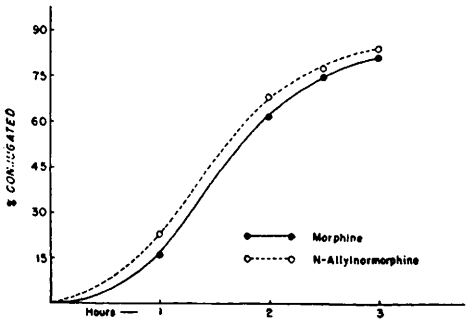

FIG. 2. The rate of conjugation of morphine and N-allylnormorphine by dog liver slices.

controls were incubated at the same time to correct for the effect of the tissue on the silicomolybdic acid reagent. At the end of the incubation time, 1 ml of 20% trichloroacetic acid was added, and after centrifuging, an aliquot was taken for the determination of the unconjugated morphine or N-allylnormorphine.

In Fig. 2 are plotted the results of these experiments. The data for each time period are the average of the results from 3 or more dogs. At the end of two and one-half hours of incubation about 75% of the morphine and N-allylnormorphine is conjugated. While N-allylnormorphine is consistently conjugated more than morphine, the difference is slight and probably not significant.

An aliquot of the solutions was hydrolyzed in 1 N hydrochloric acid by autoclaving for for one-half hour at 20 lb pressure. The average results of free morphine and N-allylnormorphine before and after hydrolysis are shown in Table I. Practically all the free base that has been conjugated can be recovered after hydrolysis.

Discussion. The use of the silicomolybdic acid reagent for the estimation of morphine must not be associated with the nitrogen atom, for N-allylnormorphine gives almost identical color intensities with the reagent. The formation of color by this reagent is therefore dependent mainly on a free phenolic hydroxyl group, for codeine does not give a color with the reagent.

TABLE I. Per Cent Recovery of Alkaloid.

	Autoclaved	
	Before	After
Morphine	35	98
N-Allylnormorphine	33	93

300 mg of tissue incubated 2 hr. Autoclaved in 1 N hydrochloric acid ½ hr at 20 lb pressure.

The conjugate that is formed *in vivo* and *in vitro* with morphine is presumed to be the glucuronide(5) but the conjugate has not been isolated or characterized. The rate of conjugation by surviving dog liver slices is the same for both morphine and N-allylnormorphine, as shown in Fig 2. Probably, the same enzyme system is responsible for the conjugation of N-allylnormorphine as is operating in the conjugation of morphine.

Conclusions. 1. N-Allylnormorphine can be estimated by the use of the Snell and Snell reagent, for a change in the alkyl group on the nitrogen does not change the intensity of the color produced. 2. N-Allylnormorphine is conjugated by surviving liver slices at the same rate as is morphine. In two and one-half hours, about 75% of the two compounds is conjugated. 3. Because conjugation of the two compounds proceeds at essentially the same rate and the effective blocking dose of N-allylnormorphine is small, the latter compound must be more firmly fixed to the receptor site than is morphine.

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