lationship with serotonin turnover in the brain." Furthermore, one might conclude that the direct method does not serve as a valid measure of serotonin turnover under some experimental conditions.

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The measurement of the serotonin [5-hydroxytryptamine (5-HT)] turnover rate after pulse injection of labeled tryptophan (TP) is derived from the following model:

$$[\mathrm{TP}] \xrightarrow[k_1]{} [\text{5-HTP}] \xrightarrow[k_2]{} [\text{5-HT}] \xrightarrow[k_3]{}$$

(where 5-HTP represents 5-hydroxytryptophan) on the assumption that x is the specific activity of TP, y is the specific activity of 5-HTP, and z is the specific activity of 5-HT.

$$\frac{dy}{dt} = k_2(x - y) \qquad (1)$$

$$\frac{dz}{dt} = k_3(y - z) \qquad (2)$$

The approximation of k_3 (k_S) that we reported (*1*) and that Hitzemann *et al.* present is derived from Eq. 3:

$$\frac{dz}{dt} = k_3(x - z) \qquad (3)$$

Equation 3 is valid only for the time interval for which the value of x is very similar to the value of y. In our report (*1*) we assumed that Eq. 3 may be used to approximate k_3 only for the time interval between 30 and 50 minutes. Moreover, we know that Eq. 3 cannot be valid throughout the entire time interval we have studied in our experiments. Indeed, the declining rate of synthesis of 5-HT calculated from our data by Hitzemann *et al.* reflects our current methodolgical inability to measure the 5-HT turnover rate. We have overcome this methodological barrier for measuring the turnover rate of heart norepinephrine (*2*), and we are currently working toward a similar method for 5-HT that will permit calculations to be made from Eq. 2.

In our report (*1*) we hesitated to give excessive weight to the specific activity of 5-HT of the control mice at 160 minutes because it did not appear to decline in this particular experiment (although it has in other experiments). Therefore, any calculations based on this particular point must be questionable. We have since repeated this experiment, using highly purified [^{14}C]TP. The conversion index (*3*) calculated for tolerant-dependent mice did not differ from that for control mice.

The probenecid and pargyline methods were excellent prototypic techniques for measurement of the rate of turnover of brain 5-HT. However, these methods do not necessarily represent ideal methods for drug studies, because they are characterized by inherent inconsistencies: pargyline interferes with the adrenocortical function (*4*), and probenecid interferes with the steady-state concentrations of brain TP (*5*).

In our report we followed the approximation of the conversion index and that of k_3, with their inherent limitations. We maintain that the rate of 5-HT turnover in the brains of mice with a high degree of tolerance to, and physical dependence on, morphine can remain unchanged. We do not exclude the possibility that one can find special strains of mice and experimental conditions in which one can obtain a change of 5-HT turnover rate associated with morphine dependence. However, the fact that we (*1*) and others (*6*) have found no association between increased turnover of brain 5-HT and physical dependence on morphine makes the idea of a causal relationship between the two phenomena highly improbable.

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The Structure of Morphine Monohemisuccinate

We have recently described the preparation and immunogenic properties of a conjugate of morphine 3-hemisuccinate with bovine serum albumin (*1*). We now have chemical evidence that the attachment of the succinoyl moiety to morphine is through the 6- rather than the 3-hydroxyl group and that the conjugate with bovine serum albumin (BSA) should therefore be similarly formulated.

We originally reported that a ferric chloride test on the succinoyl derivative (**1**) was negative. This observation has now been found to be in error; indeed **1** does give a positive test with $FeCl_3$, an indication that a free phenolic group is present at C-3 and that the succinoyl moiety must therefore be attached to the alcoholic hydroxyl at C-6. Rigorous proof was obtained as follows. Methylation of **1** with diazomethane in ether with a trace of methanol for 12 hours gave a 62 percent yield of a methyl ester methyl ether of **1** together with 7 percent of codeine, separated by thin-layer chromatography on silica gel with a solvent system composed of ethyl acetate, methanol, and ammonium hydroxide (17 : 2 : 1). The above methyl ester methyl ether on hydrolysis with 2 percent KOH in methanol at 25°C for 1 hour yielded, after one recrystallization from hexane, codeine (54 percent), which melted at 151° to 153°C and exhibited infrared

and mass spectra identical with those of an authentic sample. The above findings are compatible only with the presence of a free phenolic hydroxyl and the attachment of the succinoyl moiety via the alcoholic hydroxyl group at C-6.

The above conclusion was confirmed by the preparation of the methyl ester methyl ether of **1** from codeine as follows. Succinoylation of codeine with succinic anhydride in refluxing pyridine for 3 hours furnished codeine 6-hemisuccinate, which after purification by precipitation of its methanol solution with excess ether melted at 165° to 170°C. On methylation with diazomethane this latter acid was converted to a methyl ester, which proved identical with the methyl ester methyl ether of **1** by comparison of their infrared and nuclear magnetic resonance spectra, and their mobilities on thin-layer chromatography.

Simon, Dole, and Hiller (*2*) have described a succinoyl derivative of morphine by a procedure different from ours, which they likewise showed to be morphine 6-hemisuccinate.

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5 September 1972

Gene Therapy for Human Genetic Disease?

In contrast to Friedmann and Roblin, who state, "Storage diseases associated with lysosomal enzyme deficiencies do not appear to respond to enzyme therapy" (*1*), we believe that there is considerable hope for treatment of some lysosomal enzyme deficiencies by this means. This is so because proteins can be transported into cells through the process of pinocytosis, followed by a merger of the pinocytic vesicles with lysosomes and a mixing of their contents. Therapeutic replacement of deficient enzymes with plasma, leukocytes, or through transplanted kidneys (rather than with purified proteins which are not presently available) has already shown promise in those systemic storage disorders in which the affected cells have marked pinocytic activity (*2*).

Let us examine the two cases responsible for the pessimistic outlook of Friedmann and Roblin. In one instance (*3*), the disease selected for treatment, metachromatic leukodystrophy, may indeed be among those not amenable to enzyme replacement. In this as in other neurological disorders, the blood-brain barrier may prevent the circulating enzyme from reaching the cells of the brain.

In the second instance (*4*), plasma was infused into a patient with Fabry's disease. The purpose of the test was to monitor the relevant enzyme, ceramide trihexosidase, after it entered the patient's circulation. The quantity of enzyme was far too small to expect a notable therapeutic benefit, but could not be increased because of the deleterious effect of administering large amounts of plasma to Fabry patients. Clearly, the experiment gives no information on the therapeutic potential of purified enzyme supplied in appropriate dosage.

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Friedmann and Roblin (*1*) have been critical of the efforts of therapy for two argininemics. We are in agreement with Friedmann and Roblin concerning what must be done to satisfy any prospective techniques for gene therapy, and indeed have more than met the set criteria, as they apply to the Shope virus.

As to the criterion of prior experience with the disease, the patients were studied jointly and intensively by three groups of investigators, both from the point of view of genetics and biochemistry (*2*). The three children affected by this disease are from the same family and are of widely differing ages. The progress of the deterioration of the disease has been identical in the two older children; the third is a baby and has undergone little change as yet.

The virus used was purified by successive sedimentation in the preparative centrifuge, and subsequent separation in cesium chloride gradients and rate density gradients. It was homogeneous, as judged by electron microscopy, and produced no harmful effects on repeated blind passages in tissue culture. The virus was tested immunologically for possible contamination by other viruses, and none was found. Ultimately it was filtered through Millipore filters.

The virus has been studied for 40 years without having produced any known harmful effects on any investigator, including one who inoculated himself in 1933. Massive doses have been given to many kinds of animals without discernible effect except for, as pointed out, the decrease in blood arginine concentration. Inoculated into the skin of wild or domestic rabbits, it produces warts. The wild-type virus is not propagable in the domestic rabbit and produces no change in any tissue other than squamous epithelium. If massive doses are given the rabbit intravenously, no warts appear; only a low blood arginine develops.

The most direct evidence that the arginase induced is virus information, since the mutation of the virus is associated with a change in the structure of the enzyme, was published in 1971 (*3*). Although this evidence had been available in abstract for several years (*4*), the complete data were not available to the authors in time for inclusion in their article.

The use of the virus was said to have been perhaps premature; nevertheless, when one has a patient with a progressively deteriorating disease that is known not to respond to dietary or