TABLE II. Distribution of I^{131} in Blood Serum.

	No. of mice	Mean % of counts in TCA ppt	Range
Non-irradiated mice	28	1	.5–2.3
Irradiated mice	24	1	.2–3.8
Normal serum ppt with TCA supernatant from a radioactive mouse	12	1	.2–2.8

could be argued with some truth that in this case, since no whole protein was absorbed, the only rates measured were those of hydrolysis of protein and absorption of iodotyrosines. It is possible that the different amino acids are absorbed from the mixtures at different rates(4). Nevertheless it was thought that in this case, the iodotyrosines could be taken as typical amino acids in their absorption behavior and that the appearance of radio-activity in the blood would be a good measure of amino acid absorption in general. If significant differences in the absorption rates of normal and irradiated rats were found, they could be tken as symptomatic of the general permeability of the small intestine. Since no such differences have been found, it can be concluded that as seen in the case of fat absorption(1) no permeability differences are produced in this animal by this dosage of radiation and that in this case as well, motility and tonicity are the factors most affected. These factors have recently been investigated by Conard(5), who, using direct observation of the externalized intestine, found marked changes in motility following irradiation.

The study has also shown that no large quantities of whole protein are absorbed in either the normal or the irradiated animals, and that the ability of the irradiated animal to absorb protein in the normal fashion is essentially unimpaired for the periods and dosages studied.

Summary. The rate of protein absorption by normal mice has been measured using radioiodinated human serum albumin as a trace protein. No change in this rate could be shown after whole body X-irradiation of the mice although a change in tone and motility of the gastrointestinal tract was noted. No absorption of unhydrolyzed protein could be demonstrated.

4. Orten, A. U., Koizumi, K., France, C. J., and Johnston, C. B., *Fed. Proc.*, 1951, v10, 390.

5. Conard, R. A., *Am. J. Physiol.*, 1951, v165, 375.

Received July 2, 1951. P.S.E.B.M., 1951, v77.

Effects of N-allylnormorphine on Cholinesterases. (18904)

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Bernheim and Bernheim(1) found that morphine and apomorphine inhibit the cholinesterase of brain in sufficiently low molar concentration to enable them to speculate that this action of these drugs might be responsible for the cardiac and respiratory irregularities sometimes associated with their use. Wright and Sabine(2) confirmed the inhibitory effect of morphine on cholinesterase, and showed that Dilaudid and dihydrodesoxymorphine-D also inhibit the enzyme. They also reported that the effect of codeine varies considerably with the source of the enzyme (brain or serum). Eadie(3) reported that the inhibition of the cholinesterase of dog serum by morphine is a competitive one.

N-allylnormorphine (NAM) is a substance

1. Bernheim, F. and Bernheim, M. L. C., *J. Pharmacol.*, 1936, v57, 427.

2. Wright, C. I. and Sabine, J. C., *J. Pharmacol.*, 1941, v72, 45.

3. Eadie, G. S., *J. Biol. Chem.*, 1941, v138, 597.

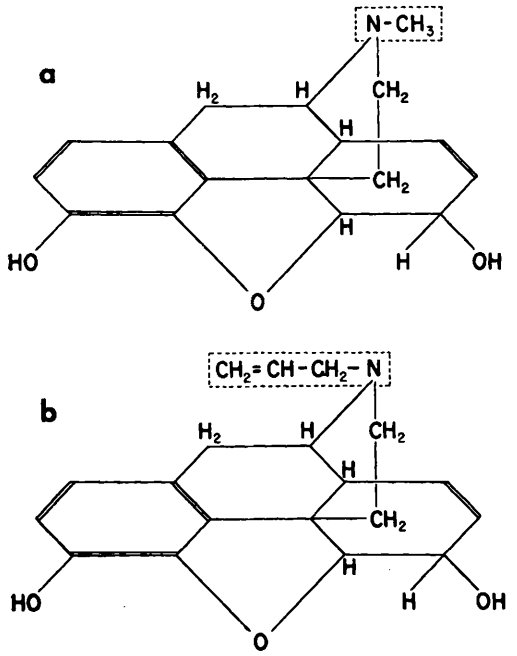

FIG. 1. a. Morphine. b. N-allylnormorphine. The dotted lines outline the different nitrogen substituents.

chemically very similar to morphine (Fig. 1), but which is antagonistic to the respiratory depressant, analgesic and stimulatory (cat) effects of morphine(4,5). Unna(4) found that although NAM abolished or prevented the analgesia due to morphine in mice, when administered alone it produced a shallow and transitory analgesia. Wikler(6) stated that in man large doses of NAM produce dysphoria, mild drowsiness, miosis, sweating and occasional nausea and circulatory disturbances.

Because of the chemical similarity and pharmacological antagonism of NAM and morphine, it was thought that NAM might be a useful tool in clarifying the relationship between the cholinesterase-inhibiting ability and the pharmacological effects of the morphine class of drugs.

Experimental. The sources of cholinesterase used were mouse brain homogenate, bovine red cell suspension and horse serum (Cutter Laboratories). The mouse brains were homogenized in Krebs-Ringer bicarbonate in a Potter-Elvehjem glass tissue homogenizer. The red cells were separated and washed three times in 0.9% NaCl, and suspended in Krebs-Ringer bicarbonate. In all cases the enzyme source was diluted with Krebs-Ringer bicarbonate so as to produce a CO_2-evolution in the uninhibited reaction of about 25 μl per 10-minute period. Variations from this value did not exceed 50%. All solutions were made up in Krebs-Ringer bicarbonate.

The enzyme and drug were placed in the main compartment of the Warburg vessel (Table I), and the acetylcholine was tipped in from the side arm at zero time. The flasks were gassed with 95% N_2— 5% CO_2. Readings were taken at 10-minute intervals for at least 60 minutes after tipping. Data were corrected for spontaneous hydrolysis of acetylcholine, possible metabolic CO_2 evolution, effects of the drugs on the enzymes and acetylcholine and thermobarometric changes.

The results of a single run are shown in Fig. 2. Only the straight-line portions of the curves are shown, since their slopes were used to calculate the percentage inhibitions presented in Table II.

Discussion. The rather wide variation in results makes interpretation difficult. This variation is believed to be a real variation in the susceptibility of the enzyme preparations to inhibition, since the agreement between the two flasks which contained the same drug in each run was always good.

There seems to be a general trend for NAM to be a slightly more potent inhibitor than morphine, but this is reversed in one case (0.0015 M on RBC).

Since NAM antagonizes most of the pharmacological actions of morphine, this seems to us to lend support to the idea that the ability of morphine to inhibit cholinesterase has little to do with its typical pharmacological effects, but that the side effects of morphine which Bernheim thought might be due to this ability, could indeed be so, since these effects are also produced by NAM.

It will be noted that the effects of these

4. Unna, K., *J. Pharmacol.*, 1943, v79, 27.

5. Huggins, R. A., Glass, W. G. and Bryan, A. R., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 540.

6. Wikler, A., *Fed. Proc.*, 1951, v10, 345.

TABLE I. Set-up for a Single Warburg Run. Final drug concentration = 0.00075 M. Flasks gassed with 95% N_2–5% CO_2.

Warburg vessel No.	Side arm		Main compartment			
	AcCh, ml	KRb	Enzyme, ml	Morphine	NAM	KRb, ml
1	.25	—	.50	—	—	2.25
2	,,	—	,,	—	—	,,
3	,,	—	,,	.75	—	1.50
4	,,	—	,,	,,	—	,,
5	,,	—	,,	—	.75	,,
6	,,	—	,,	—	,,	,,
7	,,	—	—	—	—	2.75
8	—	.25	—	—	—	,,
9	.25	—	—	—	.75	2.00
10	—	.25	.50	—	,,	1.50
11	.25	—	—	.75	—	2.00
12	—	.25	.50	,,	—	1.50
13	—	,,	,,	—	—	2.25

drugs on the serum enzyme differ quantitatively from their effects on the enzymes from brain and red cells. This is not surprising in view of the fact that many other points of difference exist between these two types of enzyme(7,8).

Summary. The inhibitions of the cholinesterases of mouse brain, bovine erythrocytes

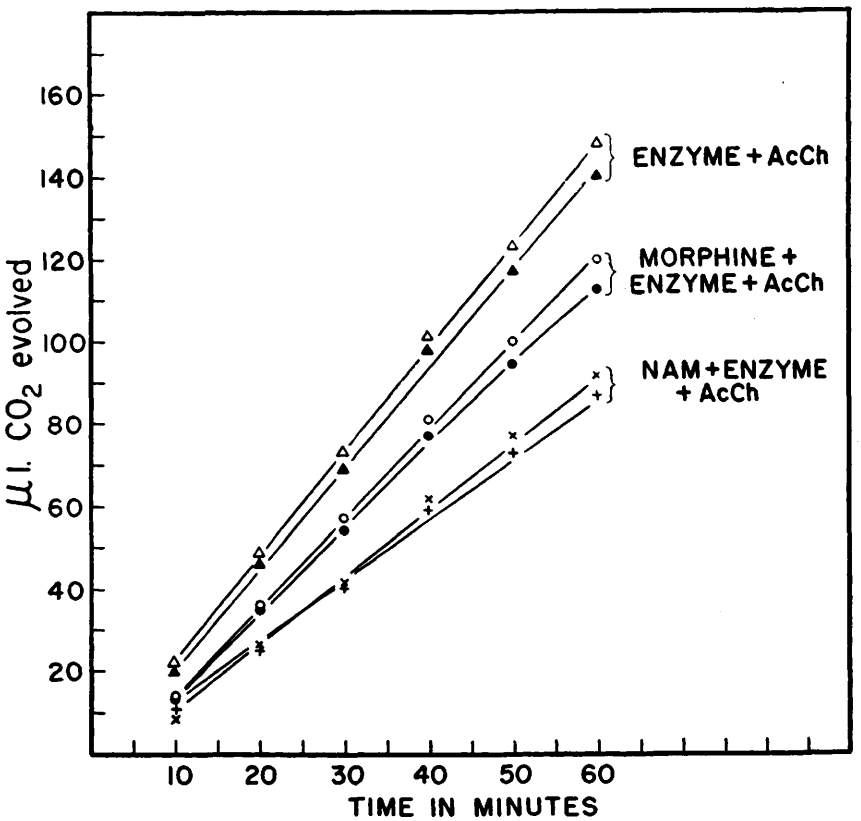

FIG. 2. Plot of results from a single run. AcCh = acetylcholine. Corrected for controls.

7. Richter, D. and Croft, P. G., *Biochem. J.*, 1942, v36, 746.

8. Nachmansohn, D. and Rothenberg, M. A., *J. Biol. Chem.*, 1945, v158, 653.

TABLE II. % Inhibitions of Cholinesterases of Mouse Brain, Bovine Red Cells and Horse Serum by Various Concentrations of Morphine and NAM. Each value represents the average of the 2 values obtained in each run.

Drug concentration	Mouse brain		Bovine RBC		Horse serum	
	Morphine	NAM	Morphine	NAM	Morphine	NAM
.00038 M	8	18	17	23	48	58
	12	21	19	23	49	60
.00075 M	33	38	24	32	50	66
	26	40	23	28	49	84
.0015 M	43	60	60	54	64	79
	44	45	35	42	54	78

and horse serum produced by N-allylnormorphine were measured and compared to the inhibitions produced by morphine. N-allylnormorphine is at least as potent an inhibitor as morphine. The implications of this finding for the theory of morphine action are discussed.

We would like to express our thanks to Dr. Russell A. Huggins, Department of Pharmacology, Baylor University College of Medicine, and Dr. Edwin L. Smith, Physiology Department of this School, for their helpful discussions and criticisms of this work, and to the Merck Co. for generously supplying the N-allylnormorphine used.

Received July 3, 1951. P.S.E.B.M., 1951, v77.

The Electron Microscopy of Rabies Inclusion (Negri) Bodies. (18905)

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The great resolving power of the electron microscope together with our present ability to cut tissue sections thin enough for examination with this instrument has made it possible to visualize several viruses within their host cells. The present paper is a record of results of a preliminary attempt to recognize rabies virus in sections of diseased mouse brain. The Negri bodies that frequently are found in such tissue have long been studied under the optical microscope without, however, their developing any general agreement on the method of their development or their relation to the virus(1-7). First attempts to see Negri bodies with the electron microscope, using a modified smear technic, have given only very limited information about them(8).

For the present experiments, 3 to 4 weeks old Swiss mice were inoculated intracerebrally with strain 1449 of Street virus (obtained through the courtesy of Dr. Harry A. Rubin of the Communicable Disease Center Laboratory, Montgomery, Ala.) and sacrificed at 7, 9 and 12 days. After removal the brains were fixed in 10% formalin. Cross sections of the cerebral hemispheres at the level of the hippocampus were hardened for 3 days in 3%

1. Acton, Hugh W. and Harvey, W. F., *Parasit.*, 1911, v4, 255.

2. Goodpasture, Ernest W., *Am. J. Path.*, 1925, v1, 547.

3. Kraus, R., Gerlach, F. and Schweinburg, F., *Lyssa bei Mensch und Tier*, Urban and Schwarzenberg, Berlin, 1926.

4. Cowdry, Edmund V., *Filterable Viruses*, Rivers, T. M., Williams and Wilkins, Baltimore, 1928.

5. Covell, W. P. and Danks, W. B. C., *Am. J. Path.*, 1932, v8, 557.

6. Nicolau, S. and Kopciowski, L., *Ann. Inst. Pasteur*, 1934, v53, 418.

7. Findlay, G. M., *Handbuch der Virusforschung*, Julius Springer, Wien, 1938, pp. 312-316.

8. Reagan, Reginald L. and Brueckner, A. L., *J. Inf. Dis.*, 1950, v87, 213.