Para-aminobenzoic acid may by itself have anti-anaphylactic properties[20] and the effect noted here might be an expression of a synergistic antianaphylactic action. It is entirely possible, however, that the decreased elimination of sodium salicylate in the urine in the presence of para-aminobenzoic acid[33] results in higher, more effective, blood levels of salicylate than when this drug is given alone. If so, other acid salts, like ammonium chloride, might also result in a comparable enhancement of the salicylate effect. While toxic reactions to salicylates and para-aminobenzoic acid may preclude the safe attainment of effective levels in the treatment of human disease, further studies are indicated to elucidate the underlying mechanisms of this protecting effect. There is reason from this and other studies to believe that a less toxic drug with antianaphylactic properties in common with salicylates might be an important therapeutic and prophylactic agent in human disease.

The now extensive studies of auto-immunization phenomena in diseases of experimental animals demand further attempts to establish the operation of such mechanisms in the pathogenesis of human disease in spite of the technical difficulties involved. It seems obvious that this phenomenon could be at work in post infectious states, the pathogenic mechanisms of which remain obscure. Further studies on rheumatic fever, post infectious leukoencephalitis, multiple sclerosis, sympathetic ophthalmia, and glomerulonephritis might, indeed, reveal that autosensitization is a common denominator for many otherwise unrelated diseases.

Summary and conclusions. 1. Progressive acute disseminated encephalomyelitis was produced in 90% of a group of guinea pigs by the subcutaneous injection of water in oil emulsions of homologous brain tissue plus adjuvants.

2. While prophylaxis and treatment with moderate dosages of either para-aminobenzoic acid or sodium salicylate alone provided no protection of guinea pigs against experimental allergic encephalomyelitis, the use of larger dosages of salicylate as well as combined salicylate and para-aminobenzoic acid therapy were effective in preventing this disease.

3. Effective prophylaxis was demonstrated only when the medication was started before or shortly after the sensitizing injection of brain tissue was given.

4. No therapeutic effect of sodium salicylate alone or combination of para-aminobenzoic acid with sodium salicylate could be shown.

33 Salassa, R. M., and Bollman, J. L., *Am. J. Physiol.*, 1948, **155**, 466.

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The Initial Uptake of C^{14}-Labelled Methadone by Rat Tissues.* (17427)

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Recently it has been shown in this laboratory that within one hour after a subcutaneous injection of 10 mg/kg of C^{-14} labelled methadone (methadone*) into rats, the concentration of the drug in the brain is relatively low.[1] A fairly rapid mobilization of the drug from the site of injection is indicated by the work of Eisenbrandt, *et al.*[2] who found that within 10 minutes after such an injection,

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1 Elliott, H. W., Chang, F. N-H., Abdou, I. A., and Anderson, H. H., *J. Pharm. and Exp. Therap.*, 1949, **95**, 494.

2 Eisenbrandt, L. L., Abdou, I. A., and Adler, T. K., *Fed. Proc.*, 1949, **8**, 288.

radioactivity appeared in the bile. In view of this rapid mobilization it was desirable either to establish or to rule out the possibility that an initial high concentration of methadone in the brain is responsible for the rather profound effects on the central nervous system.

The study was extended to include determination of the rate of accumulation of methadone* by the adrenals and the liver, both of which were found by Elliott, *et al.*,[1] to contain high concentrations after one hour, and to correlate these findings with the rate of accumulation in the blood. In addition, portions of the sciatic nerve were analyzed to check the possibility that analgesia might be produced by concentration of methadone* in the peripheral nervous system.

Methods. The C^{-14} labelled methadone HBr used in this study has been described previously.[1,3] Eight young adult rats (4 male and 4 female) of the Slonaker-Wistar strain weighing between 175-250 g and maintained on a diet of Purina Dog Chow and tap water were used in this study. Each was given 10 mg/kg of methadone* by subcutaneous injection into the left hind leg. After 10, 20, 30, or 40 minutes one male and one female were sacrificed. Ether was administered immediately before sacrifice, and a blood sample was obtained from the abdominal aorta.

Cerebrum, adrenals, liver and portions of the sciatic nerve from the uninjected (right) leg were dried to constant weight at 60°C. Tissue water content was calculated from the wet-dry weight difference.

Preparation of tissues for combustion has been described previously[1]. The method of combustion was that of Van Slyke and Folch[4] as modified by Skipper, *et al.*[5] The combustion apparatus used has been described by Entenman, *et al.*[6] Preparation of radioactive samples of $BaCO_3$ for counting was similar to that used previously in this laboratory.[1]

Although there is evidence that methadone can be metabolized by the body,[7,8] the possible metabolic products have not been identified. However, the labelled carbon atom incorporated in the methadone* used in these studies was not oxidized to CO_2.[1] Therefore, for the sake of simplicity the data are reported as micrograms of methadone*.

Results. A comparison of the concentration of radioactivity in whole blood with that in the plasma indicated a dilution of the former by the red blood cells. To establish this point definitely, the red blood cells were centrifuged, washed twice with saline and analyzed. No radioactivity could be found, thus indicating that within the period studied (between 10 and 40 minutes) the methadone* in the blood was confined to the plasma.

Furthermore, it seemed desirable to establish whether or not accumulation of the drug in the tissues was merely a reflection of accumulation in the plasma water. For this reason calculations were made on the basis of concentration per gram of tissue water in addition to the usual method of reporting such data.

The concentration of methadone* in the tissues studied is summarized in Table I. Values for the sciatic nerve are omitted from the table since at no time did the nerve contain significant amounts of radioactivity.

Discussion. The rapid appearance of radioactivity in the plasma indicates the importance of the plasma as a transport mechanism in mobilizing a subcutaneous injection of methadone.* It is of interest to note that the concentration in the cerebrum was of the same order of magnitude as that of the plasma, and also that the drug did not accumulate in the sciatic nerve. The low amounts in the cerebrum dispute the thesis that analgesia is

[3] Tolbert, B. M., Christenson, F., Chang, F. N-H., and Sah, P. P. T., *J. Am. Chem. Soc.*, in press.

[4] Van Slyke, D. D., and Folch, J., *J. Biol. Chem.*, 1940, **136**, 509.

[5] Skipper, H. E., Bryan, C. E., White, L., Jr., and Hutchinson, O. S., *J. Biol. Chem.*, 1948, **173**, 371.

[6] Entenman, C., Lerner, S. K., Chaikoff, I. L., and Dauben, W. G., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 364.

[7] Richards, J. C., and Boxer, G. E., paper presented to the Division of Medicinal Chemistry of the Am. Chem. Soc. meeting of Sept. 18-23, 1949.

[8] Way, E. L., Signorotti, B. T., and March, C. H., to be published.

TABLE I.
Tissue Concentration of Radioactivity in Terms of Micrograms of Methadone* per Gram.

Rat No.	Time after inj., min.	Cerebrum		Plasma		Adrenals		Liver	
		per g water	per g dry tissue	per g water	per g dry tissue	per g water	per g dry tissue	per g water	per g dry tissue
16	10	0	0	0.4	4.9	0	0	2.4	5.4
21	10	0	0	0.05	5.5	11.2	16.9	4.1	9.5
17	20	2.3	8.3	0.9	10.5	16.0	42.8	18.8	49.2
22	20	6.0	20.0	2.6	27.9	39.7	68.4	10.9	25.7
18	30	0	0	0.9	9.8	22.9	26.4	20.1	47.5
23	30	3.3	11.9	1.2	14.5	27.4	51.5	13.4	33.6
19	40	3.6	13.0	2.0	24.9	48.5	93.0	25.5	60.7
24	40	2.5	8.6	2.5	30.0	45.0	96.5	30.8	79.4

induced by an initial high concentration of the drug in the brain.

In contrast to the cerebrum, both the liver and adrenals showed a marked increase in concentration with time; the rate of increase in the adrenals was more pronounced. These differences, obtained even when calculated on the basis of tissue water, suggest a definite mechanism for accumulation in these tissues.

Summary. Based on the determination of radioactivity for periods of 10, 20, 30, and 40 minutes following a subcutaneous injection of 10 mg/kg of C^{-14} labelled methadone HBr into rats, it was found that:

(a) Methadone* makes its appearance in the blood within 10 minutes, and is confined to the plasma.

(b) There was no activity in the cerebrum before 10 minutes, and even thereafter the concentration is very low. The rate of accumulation roughly parallels that of the plasma indicating no special affinity of the brain for the drug.

(c) The adrenals and liver show a marked ability to accumulate methadone* at a rate in excess of that shown by either the cerebrum or plasma.

(d) There was no activity in the sciatic nerve within the period studied.

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A Nervous Syndrome Produced with Phenylalanine and Methionine.* (17428)

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During studies on the metabolic interrelationships between threonine, phenylalanine, tryptophan and niacin, it was noted that animals fed a niacin-tryptophan deficient ration supplemented with 0.208% DL-phenylalanine and 0.2% DL-methionine for a period of 12 weeks developed an interesting nervous syndrome. The symptoms include an arched back with extended legs in a rigid condition. The head oscillates vertically almost continuously, and the rate of movement increases until the animal apparently becomes fatigued. After a short rest period, this movement reoccurs. The animal may show a slow rotary motion of the body and periodically it may move backwards at a rapid rate. Death re-

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