view of these facts we have previously postulated that "it is not unlikely that a drug-induced increase in activity of normal brain may inhibit discharges from seizure foci"(20). The greater ability of Benadryl, as compared with Pyribenzamine, to stimulate the central nervous system in subconvulsive doses could account for the salutary effect of this drug in petit mal.

Summary. Benadryl, Pyribenzamine and Dilantin were tested in rats for toxicity and for ability to modify maximal electroshock seizure pattern and to prevent Metrazol convulsions. The data indicate that all three drugs modify maximal electroshock seizure pattern but do not prevent Metrazol convulsions. The significance of the data is discussed.

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Influence of Methadone on Oxygen Uptake and Glycogenolysis of Rat Liver Slices.* (18158)

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The effects of morphine-like analgetics, including methadone, on the metabolism of surviving tissues and isolated enzyme systems have been studied by several workers(1-4), in an attempt to explain the mechanism of action of analgetics. Although these studies have not accomplished their primary purpose, they show that analgetics affect tissue metabolism in a manner different from anesthetics, and they indicate the usefulness of methadone as a tool in the study of oxidative processes.

The present study was undertaken as a result of the observation that the same concentration of methadone increased the oxygen uptake of liver slices from fed rats but decreased that of slices from rats starved overnight. Since the effect was the same whether or not glucose was included in the suspending medium, the possibility of increased glycogenolysis by liver slices in the presence of methadone has been investigated by biochemical and histological techniques.

Methods. Liver slices were obtained from adult albino rats of the Slonaker-Wistar strain. When high initial liver glycogen values were desired, the animals were starved for twenty-four hours and then given a mixture of half glucose, half ground stock diet overnight before use. Animals which had been starved overnight provided liver slices low in glycogen content. The rats were sacrificed by a blow on the head, the livers quickly removed, placed in a cold box(5) and with a Martin slicer(6) cut into slices approximately 0.5 mm thick. The slices were randomized and used either for measurement of oxygen uptake, chemical determination of glycogen or histologic examination. Oxygen consumption was measured by the direct method of Warburg(7) at 37.2°C. The liver slices which weighed 50-100 mg were suspended in 2 ml of a modified Krebs-Ringer phosphate buffer(4) with or

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without 0.2% glucose. (Extracellular Ringers = ER; with 0.2% glucose added = EGR.) Vessels containing tissue from either fed or starved animals were read at 15-minute intervals for 90 minutes to establish control QO_2's for each vessel. Then to half the vessels 0.005M methadone was added from the side arms of the Warburg flasks to make a final concentration of 0.0005M, and readings were continued for an experimental period of 90 minutes. The quantitative determination of glycogen was done by the method of van Wagtendonk *et al.*(8). Analyses were performed at death, at the time the Warburg vessels were placed in the constant temperature bath, and at the completion of each 180-minute run.

Liver slices from 2 fed animals were used for histologic examination of glycogen. No starved animals were included because the low glycogen content of their livers would make histologic differentiation of glycogen difficult. For the same reason, the 90-minute control period was omitted in the determination of oxygen uptake; methadone was added to half the vessels at zero time and QO_2's were measured on all vessels for a 90-minute experimental period. Control slices were fixed at the time the Warburg vessels were placed on the 37.2°C bath; all others after they had respired for 90 minutes in EGR with or without methadone. The fixative was ice cold Rossman's picro-alcohol-formalin mixture(9). After fixation 8 μ celloidin sections were stained with hematoxylin and Best's carmine and mounted.

Results. Oxygen Uptake. QO_2's were determined by the direct method of Warburg on liver slices from 10 fed and 6 starved animals. When the data were analyzed, it was apparent that the presence of glucose in the Ringer solution did not alter the QO_2's of either control or experimental slices; hence the results were combined and are presented graphically in Fig. 1. The oxygen uptake of slices from fed animals was increased immediately to a maximum of 127% of the control value at 120 minutes, falling to 119% at 180 minutes; the latter value was found to be significant by comparing probable errors of the means ($P<0.001$). In contrast, methadone significantly inhibited the oxygen uptake of slices from starved animals; at 180 minutes the experimental value was 51% of the control ($P<0.001$). The higher control values for slices from starved as compared with fed rats may be attributed to the increased bulk of the fed livers resulting from the storage of glycogen and water(10), *i.e.*, metabolically inert weight.

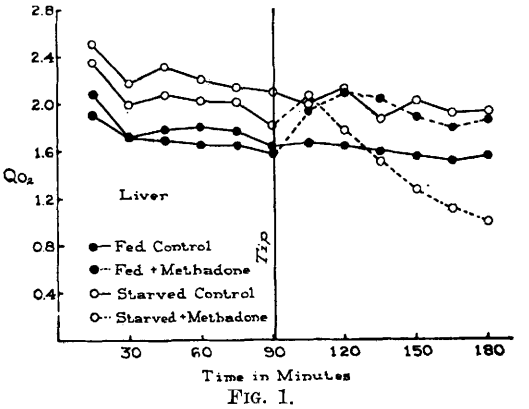

FIG. 1.

The effects of methadone on the oxygen uptake of liver slices from fed and starved rats. Each point represents the average of 29-39 slices from 10 animals in the case of the fed animals and 24 slices from 6 starved animals.

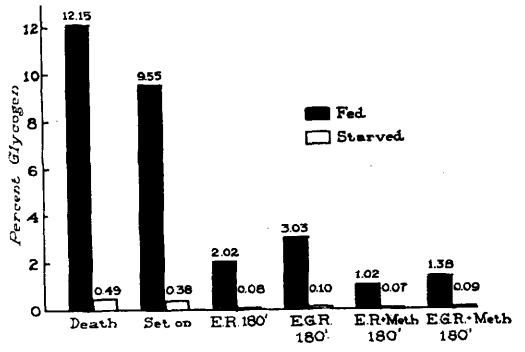

FIG. 2.

The glycogen content of liver slices from fed and starved animals. See text for explanation.

Glycogen Analyses. The results of the glycogen analyses are graphically portrayed in Fig. 2. Overnight starvation depleted liver

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glycogen to relatively low values. Livers from 6 fed animals contained 12.15 ± 0.91% (PE mean) glycogen falling to 9.55 ± 0.62% in the 20-30 minutes required to prepare the slices for oxygen uptake studies. In the control vessels at 180 minutes glycogen content fell to 2.02 ± 0.12% in ER and 3.03 ± 0.18% in EGR. The glycogen sparing effect of glucose was significant ($P = 0.001$). Methadone (0.0005M) significantly increased the rate of glycogenolysis in ER or EGR ($P<0.001$ in both cases). The glycogen content of methadone treated slices at 180 minutes was 1.02 ± 0.15% in ER and 1.38 ± 0.16% in EGR. The glycogen sparing effect of glucose was found to be not significant ($P = 0.25$), but the trend was the same as in the control vessels.

Histologic Examination. Sections prepared from tissue which had respired in EGR for 90 minutes showed partial depletion of glycogen throughout some and peripheral exhaustion in all; glycogen was absent from the outer one or two cell layers. In the presence of methadone marked glycogen depletion was obvious throughout all sections and peripheral depletion extended deeper than in control slices. No damage to cellular structure could be detected.

Discussion. The ability of the same concentration of methadone to inhibit the oxygen uptake of liver slices poor in glycogen and increase the oxygen uptake of slices rich in glycogen suggests that the observed stimulation is actually a manifestation of enzyme inhibition. According to McElroy(11), it is generally accepted that, in various organisms, if oxidative phosphorylation is inhibited, inorganic phosphate will increase to a concentration which will permit a rapid breakdown of stored material such as glycogen. The fact that under the conditions of our experiments the glycogen sparing effect of added glucose is apparent in the absence of methadone and is practically obliterated by 0.0005M methadone, coupled with the finding that methadone can inhibit hexokinase(1) suggests that such a mechanism may be operating here. However, such a succinct explanation must be viewed with caution since a large excess of inorganic phosphate is always available in the suspending medium. In addition, it has been shown that methadone increases the oxygen uptake of rat brain slices respiring in a medium containing glucose(4).

Preliminary experiments indicate that in bicarbonate buffer 0.0005M methadone does not stimulate glycogenolysis and inhibits oxygen uptake; hence, further studies are contemplated to investigate the role of ionic constituents of the suspending medium in producing the findings reported above.

Summary. 1. Methadone (0.0005M) increases the oxygen uptake of liver slices rich in glycogen and depresses it in slices poor in glycogen. 2. The increased oxygen uptake is accompanied by an increased rate of glycogenolysis as demonstrated by biochemical and histologic methods. 3. A possible explanation for the findings is discussed.

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