of active sites, physico-chemical studies of secondary and tertiary structure, amino acid sequence studies, to mention only a few possibly dangerous instances. In our studies(1, 2) where small amounts of active and inactive radiation products from egg-white lysozyme must be isolated, the necessity for enzyme homogeneity is immediately obvious. Our studies of radiation effects, at present have been limited to those on the major chromatographic component from crystalline egg-white lysozyme. The availability of the minor chromatographic components in gram amounts from our many preparative columns now affords the opportunity to study radiation effects in these materials with a view to assessing the radiation lability of closely related protein structures.

Summary. Seven components have been demonstrated chromatographically in crystalline preparations of egg-white lysozyme. This was effected with a chromatographic procedure employing BioRex 70 columns eluted with 0.2 *M* potassium phosphate buffer, pH 7.09. Six components possess enzymic activity and 3 have molecular weights very close to that of the major chromatographic component. The potential danger of assuming homogeneity in crystalline lysozyme preparations is indicated, particularly in regard to the study of radiation products.

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Anti-Alcohol Properties of Metronidazole in Rats.* (31698)

BERRY CAMPBELL, JOANN T. TAYLOR, AND WILFORD L. HASLETT
University of California, California College of Medicine, Los Angeles

It is known that certain laboratory animals will freely imbibe weak solutions of ethyl alcohol in preference to water, such preference varying from species to species(1), and within species according to genetic constitution(2-5), nutritional state(6-11), metabolic factors(12) and conditions of stress(13-18); there is even some evidence(19) that individual personality traits (*i.e.* timidity) may determine an animal's drinking pattern.

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The most thoroughly studied species in this regard is the rat. It has been found that some rats naturally prefer alcohol solutions to water. If they do not, a preference may be induced by simply restricting their fluid intake to an aqueous solution of alcohol for an extended period(20); the different degrees of preference which develop appear to be a function of the length of time the animals consume alcohol, and not of the particular concentration consumed(21). Preferences for

much higher concentrations have been induced by infusion of the cerebral ventricles with alcohol solutions(22).

The phenomenon has been referred to as a habituation and has been analogized cautiously with the true alcoholism seen in humans. As such, numerous investigators have utilized these findings as a tool in examining the effect of various pharmacological agents upon alcohol consumption. Thus, amphetamine sulfate (23), alloxan(24) and estrogens(25) decrease alcohol intake, while meprobamate(22,26), hydroxyphenamate(22) and insulin (and the insulin-like compound, Nadisan)(24) increase it. Conflicting results have been reported with thyroid preparations(27,28).

The experiments reported here were performed in an attempt to confirm, experimentally, the clinical observation(29) that Metronidazole (Flagyl), a trichomonacide(30), possesses anti-alcohol activity.

Sprague-Dawley rats of either sex, each initially weighing between 250 and 300 g, were individually housed in rack-mounted, hanging cages constructed of stainless steel mesh. Each cage was provided with 2 identical 100 ml bottles which dispensed fluid through stainless steel nipples.

All rats were allowed 10 to 15 days in which to acclimate to their new surroundings. During this time, both bottles in the cage contained water and a routine was established whereby fluid intake was measured at the same hour daily. This period was followed by 20-48 days of free choice, during which each animal was allowed to choose either water or a solution of 5% ethanol. Bottles were alternated daily during this phase of the experiment, and throughout subsequent free choice periods, to prevent the position of the bottles from influencing choice(31). The future course of treatment for each animal was determined by the drinking pattern established during this initial free choice period. The amount of fluid consumed by each animal was recorded daily by measurement of the residue in each bottle.

All animals were maintained on an *ad libitum* diet of Purina Mouse Breeder Chow. The rat quarters were under continuous illumination and were held at a temperature of 21-24°C.

Metronidazole was administered intraperitoneally (I.P.) in a total volume of 1-1.25 ml as a suspension (50 mg/ml) in physiological saline. Control injections consisted of an equal volume of physiological saline alone.

Several drinking patterns, previously described by other authors(26), were discernible during the initial free choice period. About one-third of the animals immediately consumed 75% or more of their daily fluid in the form of 5% ethanol. In contrast, another one-third consumed 25% or less of the ethanol solution. However, some of these latter rats developed a stable preference for the 5% ethanol if allowed to remain on the free choice regimen for a long enough period of time (up to 40 days). The preference of remaining animals lay either midway between these two extremes or no preference was exhibited.

In the first experiment, 18 male rats were observed for 20 days on free choice. They were then subjected to a 65-day "habituation" period during which 5% ethanol was their only available fluid. When again observed on free choice, 11 of the 18 rats showed a consistent increase in the ratio of 5% ethanol consumed/total fluid consumed. These rats were selected for further observation and designated Group I. They were then given daily Metronidazole injections (125 mg/kg) for 14 days while on free choice. After withdrawal of the drug, free choice was continued for 2 weeks. Fig. 1 shows the average daily intake of this group. It can be seen that a significantly higher intake ratio (indicating increased alcohol consumption) follows the habituation period, and that drug treatment dramatically depresses it to pre-habituation levels. Withdrawal of Metronidazole was accompanied by a slight, gradual rise in the daily ratio of alcohol intake of 3 of the 11 animals. The total alcohol consumed by this series fell from approximately 25 ml/day to 12 ml/day following the drug administration.

This group of 11 animals was readdicted for 28 days, then placed on free choice for 44 days. The drug treatment was then repeated, this time preceded by a 9-day period during which daily injections of physiological saline were given. No significant drop in alcohol consumption was seen during the saline control period, while a precipitous drop again

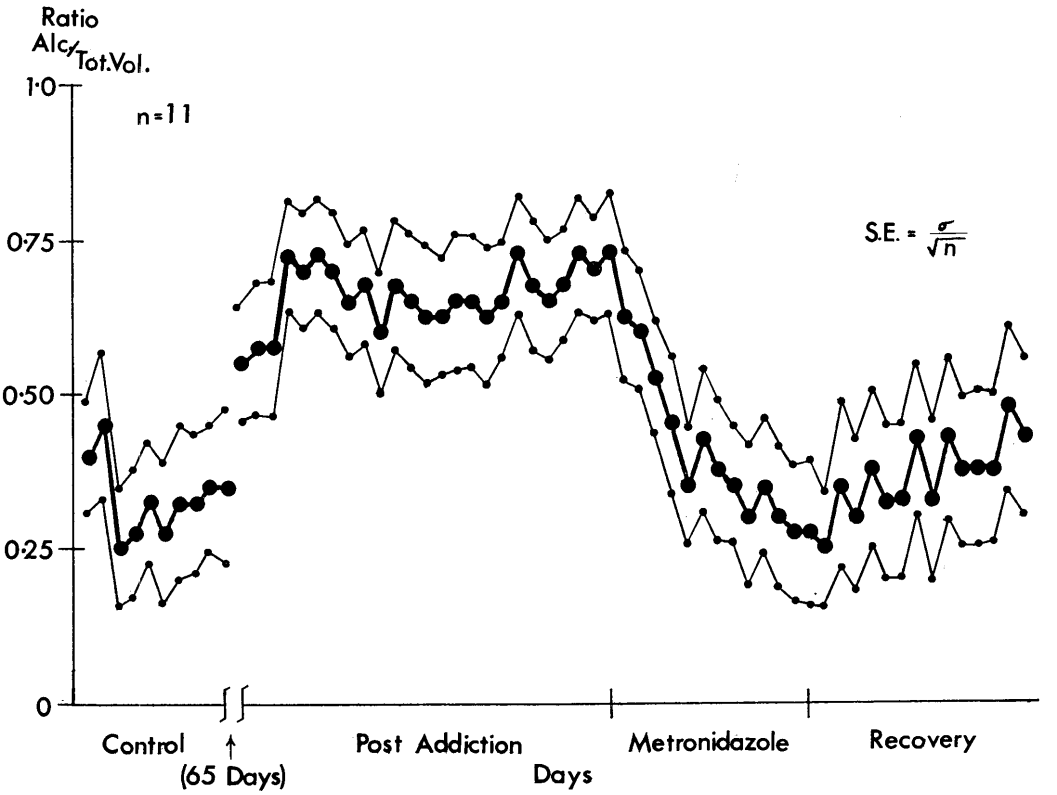

FIG. 1. Average daily alcohol intake of 11 rats of Group I. Alcohol consumption is expressed as the ratio of ml of 5% ethanol to ml of total fluid consumption. The first 10 days of observation are succeeded by a 65-day habituation period in which 5% ethanol alone was available. After a post addiction period of free choice, these rats were followed through 14 days of drug administration and through a subsequent recovery period. The standard error of the mean is charted in the enveloping curves. Each point represents a daily value.

resulted from the injections of Metronidazole.

A second group of rats was observed for 48 days on a free choice regimen. From this group, 10 male and 8 female rats were selected whose preference for 5% ethanol, with no habituation period, exceeded 75% of their total daily fluid intake. These 18 animals were designated as Group II. The 48-day free choice period was followed by 7 daily injections of saline, and then by a 21-day regimen of Metronidazole (125 mg/kg). The average decline in alcohol consumption (Fig. 2) was similar to that seen in Group I. Six relatively unresponsive animals from this group were given 3 times the dose of Metronidazole (375 mg/kg) for an additional 14 days. The high addiction pattern in these rats remained unaltered. To test the possibility that sex differences were important in the response to this agent, the data were segregated and analyzed separately for the sexes. There is no indication of a significant difference between the male and female rats.

These experiments have shown that Metronidazole (125 mg/kg) depresses the ratio of 5% ethanol consumption/total fluid consumption in rats which prefer 5% ethanol to water. This drug was effective both in those animals which exhibited a natural preference for ethanol and in those which had been forcefully habituated. However, some of these rats retained their preference for 5% ethanol even in the presence of very high doses of drugs (375 mg/kg); these resistant animals were distributed between the naturally and forcefully habituated groups.

No attempt has been made to correlate the alcohol preference shown by rats to the alcoholism seen in humans. However, the drinking patterns shown by the rats in the present

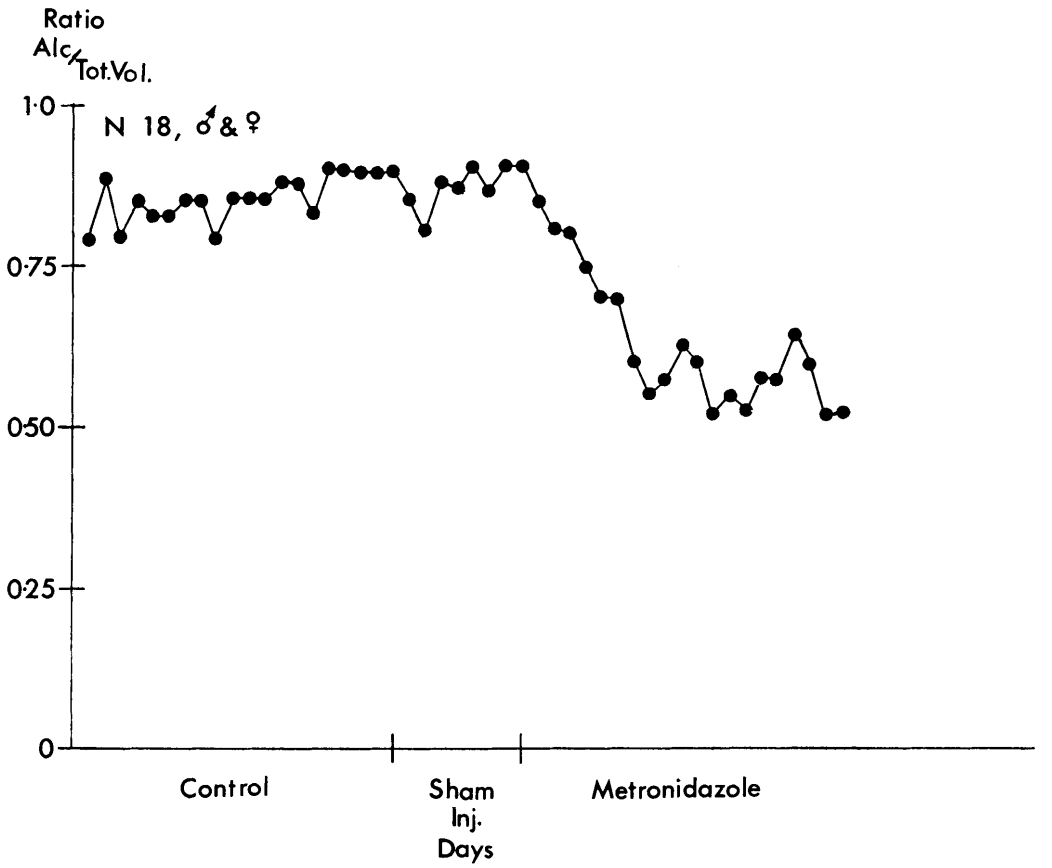

FIG. 2. Average daily alcohol intake of 18 rats of Group II, expressed as the ratio of ml of 5% ethanol to ml of total fluid consumption. Each point represents a daily value. The 48-day control period in which the rats were on free choice of fluid was succeeded by a period of 7 days in which they received shown injections. They were then observed through a 21 day period of drug injection.

experiments resembled human drinking habits, in that there was great individual variation, not only in the amount of alcohol accepted spontaneously, but also in the ease with which habituation was induced through enforced alcohol intake. Similarly, as with humans, the rats varied in their response to drug therapy.

Although the metabolic phenomena underlying these effects are unknown, there is a possibility that Metronidazole exerts its effect through inhibition of alcohol dehydrogenase, acetylaldehyde dehydrogenase, xanthine oxidase, and synthesis of thyrotropic substances. These relationships have been discussed elsewhere(32).

Summary. The effect of Metronidazole upon alcohol consumption of two groups of rats has been measured. The first group of 11 animals was selected because they clearly showed a heightened preference for alcohol after a period of forced addiction. In these, a daily intraperitoneal injection of Metronidazole was followed by a precipitous drop in alcohol consumption. In a second group, 18 animals selected because of their primary preference for alcohol, demonstrated the same substantial drop in alcohol consumption after the Metronidazole injections. No sex differences were found.

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Water-Food Interrelationships in Rats Fed Chemically Defined Liquid Diets.* (31699)

R. SHAPIRO, B. K. GOLD, AND N. A. ROSENTHAL (Introduced by P. Griminger)
Schwarz BioResearch, Inc., Orangeburg, N. Y.

Most studies concerned with thirst, water metabolism and water-food interrelationships have been conducted under conditions of separate feeding of food and water. Under these conditions quantitative relationships between food intake and water intake have been demonstrated and show that restriction of water intake in normal rats depresses food intake and restriction of food intake reduces water intake(1,2).

In contrast, relatively few studies concerning food-water interrelationships have been conducted when both were administered as a single formulation. Adolph and Parmington (3) showed that when food and water were fed together as milk, the water intake was governed by calorie content alone, even though this required ingestion of excess fluid. Feeding a similar milk diet to rats with diabetes insipidus Bruce and Kennedy(4) also showed that food intake depends on caloric content but water intake ultimately depends on the demands imposed by the renal excretion of the products of metabolism. Both studies were of short duration (7-10 days) and both employed extremely dilute diets (less than 10% solids).

Greenstein *et al*(5) developed water soluble completely defined liquid diets containing synthetic amino acids, glucose, vitamins, minerals and ethyl linoleate. Such diets could be useful for studying water-food interrelationships when the two constitute a single physical mixture. The following experiments were

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