THE EFFECT OF THE NUTRITIVE VALUE OF BUTTER FAT AND CORN OIL RATIONS ON THE GROWTH AND THE MAZE LEARNING ABILITY OF ALBINO RATS

by

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INTRODUCTION

With the widespread use of vegetable oils in place of butter has come the question of the comparative nutritive value of these fats. There is special interest as to whether "filled" milk, milk which has had the milk fats removed and vegetable fats added, is as healthful as ordinary milk. If this substitution of fat is harmful in any way to animals such as rats, it also might be harmful to human infants. Considerable research has been done on the problem, mainly from the standpoint of the effect upon growth, but results have varied considerably. This investigation was carried out with the purpose of extending the information already available on this subject. The experiment was designed to study the effect of corn oil and butter fat on the growth of albino rats and to find out if the two rations had different effects upon the maze learning ability of the rats. The latter problem is of interest because of the bearing it might have upon the nutrition and development of children.

Review of Literature

In 1932 Issachsen (20) reported that artificial milk, centrifuged milk containing finely dispersed plant fat and cod-liver oil, did not exert as favorable an influence in the course of digestion as whole milk. Pigs were used in his experiment.

The following year an experiment (23) on white rats was reported which compared the nutritive value of margarine and soybean oils with butter fat. The fat was added in such a concentration that 30 per cent of the caloric value of the ration was due to fat. Rats fed butter fat and soybean oil rations showed the same increase in weight, while the margarine rats gained more slowly. When these rats were subjected to starvation, the soybean oil animals lived the longest. The high nutritive values of margarine and soybean oil were thought to be due to the high content of unsaturated fatty acids in these oils.

In 1940, Schantz, et. al., (27) of the University of Wisconsin, came out with a report on their experiments. They used weanling rats at 21 days of age, placing them on rations of fresh skim milk into which fat had been homogenized to give a fat content of 4.0 per cent. Minerals and vitamins were added. The various fats used were butter fat, corn oil, coconut oil, cottonseed oil, and soybean oil. Feeding was ad libitum in amounts not greatly exceeding daily consumption, which helped to keep the fat from separating. The butter fat ration consistently gave better results, and the data on growth were statistically significant. Gains in relation to food consumption were more efficient for the butter fat ration than for the others. Next they added some of the non-saponifiable portion of the butter fat to corn oil. The rats fed on this corn oil showed no better growth than on corn oil alone. Thus it appeared that the growth-stimulating property of butter fat was in the saponifiable fraction or was destroyed during saponification.

Following up the last lead, they reasoned that the phospholipids, which decompose on saponification, might be the growth factor in butter fat (25). This time egg lecithin was added to corn oil to the 0.25 per cent level. This ration promoted better growth, but the "enriched" corn oil was still not as good as butter fat. Egg lecithin added to the 0.5 per cent level produced no greater improvement. Additions to corn oil of sphingomylin, sphingosine sulfate, ethanolamine, and choline were made, but gains on these rations were in no case as good as with butter fat. The addition of choline seemed to improve the corn oil slightly in the case of the females. From this experiment it appeared that something other than phospholipid content of butter fat was responsible for stimulating growth.

Still in search of the growth-stimulating factor of butter fat, Schantz, et. al., (26) divided the fatty acids of butter into volatile, unsaturated, and saturated fractions. The glycerol esters of these fractions were then added to the corn oil to be used in the rations. The various fractions were added in approximately the proportions they were found in butter. The rats on the corn oil ration containing the saturated fatty acid fraction gave better growth than rats grown on butter fat ration. The efficiencies of the gains as compared to the food consumption were the same for these two groups of rats. From this experiment it appeared that the superiority of butter fat was due to the appearance of long-chain saturated fatty acids.

Boutwell, et. al., (2) of the same laboratory, made rations in which (a) the hydrogenated, saturated fatty acid fraction, (b) the unhydrogenated, saturated fatty acid fraction, (c) the hydrogenated, unsaturated fatty acid fraction, and (d) the unhydrogenated, unsaturated fatty acid fraction were added to corn oil. Rats which received the corn oil ration containing the hydrogenated, unsaturated fatty acids grew better than rats on any other ration. However, the hydrogenation of corn oil didn't increase the growth produced by the corn oil. From this it was concluded that the unsaturated form of the growth-promoting, saturated fatty acid must not be present in the vegetable oils, whereas it is relatively high in butter.

In further experimentations, Boutwell, et. al., (3) used rations of extracted skim milk powder. Fat as butter fat or corn oil was added to the 25, 30, and 35 per cent levels. Vitamins and minerals were added. The rats showed the greatest weight gain difference in six weeks between the butter fat and corn oil rations when the fat was added at the 35 per cent level. Rats 14 days, 21 days, and 30 days old were started on these rations. The youngest animals showed the greatest weight gain differences between groups. If lactose was the only carbohydrate in a ration of casein, carbohydrate, and fat, a greater difference in growth was shown by the butter fat and corn oil rations than if the carbohydrate of the ration was other than lactose or a mixture of carbohydrates.

Except in the preceding experiment, the Wisconsin workers had used rations in which fat, vitamins, and minerals only were added to the skim milk or skim milk powder. The only carbohydrate in the ration was the lactose of the skim milk. In this experiment (16), lactose was added to bring the carbohydrate content up to the 32 per cent and 48 per cent levels. It was found that the growth difference between rats fed on butter fat and corn oil rations was greater on the 48 per cent lactose ration than on the 32 per cent lactose ration. It was concluded that lactose had some effect on the intestinal condition of the rats which was counteracted by butter fat but not by corn oil.

Boutwell, et. al., (4) ran an experiment using two series of carbohydrate rations. These rations were composed of 48 per cent carbohydrate (as lactose or a mixture of lactose, sucrose, dextrose, starch, and dextrin), 28 per cent fat (as butter fat, corn oil, coconut oil, soybean oil, peanut oil, olive oil, Crisco, or cottonseed oil), 20 per cent casein, and 4 per cent salts, with vitamins added. In all cases growth on each of the fats was greater for rats fed on the mixed carbohydrate rations, and the differences in growth between the carbohydrate rations were more pronounced in the case of the vegetable oils. There was no marked difference in efficiency (ratio of weight gain to feed consumption) of groups within the mixed carbohydrate series, though the efficiency was greater for the mixed carbohydrate series than for the lactose series.

While these investigations were under way, other investigators were working along similar lines. Harris and Mosher (17) used rations of 25 per cent fat added as coconut oil or butter fat, 72 per cent extracted milk powder, and 3 per cent extracted yeast. Vitamins and minerals were added. The rats were started on these rations at 120 days. The first 30 days growth on the two rations was about the same. After that, the rats on the coconut oil ration grew more rapidly. The food consumption, however, was less. There was no evidence of pathological tissue change in any animal, which indicated that, on that high a fat content, these fats were equally harmless to the animals.

Richter (24) used survival time and activity of rats as measures of the nutritive value of the fats. He found that the fats gave decreasing survival time in the following order:

butter, olive oil, lard, wheat germ oil, cod liver oil, Crisco, peanut oil, perilla oil, and glycerine. The rats had the greatest appetite for butter, olive oil, and lard and smallest for perilla oil and glycerine. According to Richter, the daily intake of a single food, as in this experiment, will depend upon (a) taste, (b) digestibility, (c) absorption, (d) satisfaction, and (e) excretion.

Hoagland and Snider (19) fed rats on rations of varied fat content and collected the feces for a week. The fat content of the feces was determined. Then the rats were transferred to fat-free diets. The feces were collected again for a week after the rats had been on the fat-free rations for several days. The fat content of these feces was assumed to be metabolic fat. This made it possible to determine the amount of fat actually used by the rat. The amount of fat used was transferred to a digestibility coefficient by dividing by the amount of fat consumed. It was found that the growth-promoting value of a fat did not show a consistent relationship to its digestive coefficient. Also, there was no apparent relationship between the chemical composition of a fat and its nutritive value. Cottonseed oil diets were much poorer at a 30 per cent level than at a 5 per cent level.

Euler, Euler, and Saberg (12, 13) conducted experiments in which the fat portion of the rations was butter and margarine. They found that rats fed on the margarine ration gained more in the six weeks of experimentation, had a better appearance, and seemed stronger than the rats fed on the butter ration. They found that during the first three-weeks period the margarine rats grew 9.4 per cent faster than the butter rats, and the second three-weeks, they gained 40 per cent faster. When no vitamin D was added to the diet, the butter rats were superior.

Freeman and Ivy (15) used diets that they obtained on the open market--evaporated milk and "milk" (evaporated skim milk) containing coconut oil. Growth was somewhat greater on evaporated milk, but the difference was not significant at 49 days. At 97 days, the difference was significant. The difference couldn't be ascribed to any particular aspect of the diets, however, as they differed in solids, nitrogen content, and ash as well as having different fats present.

Deuel, et. al., (5, 8, 9, 10) used rations of 68 per cent skim milk and 32 per cent fat, the fats being butter fat, corn oil, vegetable margarine, cottonseed oil, olive oil, peanut oil, and soybean oil. No difference in growth was found at three or six weeks or for the males at twelve weeks. At twelve weeks the female rats on butter fat ration weighed slightly, but not significantly, more. When diacetyl was added to the vegetable fats, there was no difference noted at any interval for either male or female rats. Also, the bone growth of the groups was equal, as measured by x-ray of the tibia length. There were no significant differences in water, protein, lipid, ash, and calcium content of the rats, so it was assumed that the rations allowed similar tissue growth. There were slight differences in the composition of the female rats and that of the male rats. The efficiencies of conversion to body tissue for the various fats were the same. No difference was observed in fertility of male or female rats fed on the various rations. Deuel, et. al., explain the discrepancies between their experiment and other workers using ad libitum feeding as due to weanling rats preferring butter flavor so that they consumed more of the butter fat feed.

Deuel and Movitt (6) tested the food preferences of rats, using the same diets as before. The rats, given a choice, consumed the butter fat ration to the greatest extent. When diacetyl or commercial butter flavor was added to the vegetable fat rations, consumption was increased.

Ershoff and Deuel (11) conducted an experiment on various carbohydrates for use in the ration. Lactose, beta-lactose, galactose, glucose, sucrose, corn starch, and half and half beta-lactose and sucrose were used at the 73.2 per cent level in rations of casein, vitamins, and salts. The rats also received a daily supplement of corn oil. Rats of the Long-Evans strain and of the University of Southern California strain, a modified Wistar, were used. The rats on beta-lactose developed a syndrome of severe diarrhea, unthrifty appearance, ruffled fur, edema of the hind paws, alopecia, and finally death. Rats on lactose had a similar condition but survived longer. The University of Southern California rats survived longer on both rations than did those of the Long- Evans strain. Since Wisconsin workers (4, 16) had suggested a possible interrelationship between the kind of carbohydrate and the kind of fat, the corn oil was omitted from the diet and butter fat, oleomargarine, or lard was incorporated to the extent of 10 per cent of the diet, replacing a similar quantity of the carbohydrate. This change of fat was not effective in preventing the appearance of the syndrome. The condition was thought to be due to an alternation of the intestinal flora from the proteolytic to the acidophilic type, since lactose brings about changes in pH in the intestive which favors the growth of the acidophilic type. This was thought to cause either a failure of intestinal synthesis or increased utilization of factors already present.

Deuel, et. al., (7) gave, as possible explanations of the difference in efficiency of the rations used by them and by Wisconsin workers, (a) the lower proportion of lactose in the California ration and (b) the use of different strains of rats.

Zialicita and Mitchell (28) used a paired-feeding method for studying corn oil and butter fat rations. Some of the rats were weaned at one week, others were weaned at two weeks. A liquid ration was used until the rats were three weeks of age, than a solid ration was fed. The solid ration was composed of extracted skim milk powder, casein, fat, a salt mixture, and vitamins. There were no significant differences in gains in weight, or in chemical composition, except in ether extract and gross energy content of the rats weaned at two weeks. Using <u>ad libitum</u> feeding, they found that the rats fed on corn oil made greater gain and obtained greater body length. They also consumed more feed. The differences were significant in food consumption, body weight, and body length. Zialicita and Mitchell concluded that, apart from vitamin content, corn oil and butter fat were essentially the same in growth-promoting value for the rat.

The Committee on Fats of the Food and Nutrition Board, National Research Council, (14) summed up the information on the subject as follows:

> There is no complete agreement in the results from the different laboratories as to the comparative value of different fats fed at the same level. Furthermore, with repeated experiments the results are not always the same in a given laboratory. Differences between fats will be found at one time which are statistically significant but which are not reproducible at another time with a new group of rats and a new batch of the same kind of fat.

Although it may be seen considerable work has been done on the nutritive value of fats, no two groups of investigators have used the same type of rations in their studies. In view of the conflicting data reviewed above, it was decided a study should be undertaken for the purpose of further investigating the difference in the growth-promoting effect of corn oil and butter fat when included in the high-lactose, skim milk ration used by Geyer, et. al., (16) and to determine whether these rations produced different effects on the maze learning ability of albino rats.

GROWTH EXPERIMENTS

Procedure

The albino rats used for the growth studies were obtained from Sprague-Dawley, Madison, Wisconsin, or from litters raised in this laboratory. Following weaning at 20 to 21 days of age, the rats were divided as evenly as possible into two groups by weight and each of these groups was put on one of the experimental feeds. Since Wisconsin workers (3, 16) found that more distinct growth differences developed in rats fed on butter fat and corn oil rations if lactose was the sole carbohydrate and that the higher the percentage of lactose the more distinct the difference, it was decided that the same high-lactose rations should be used for this experiment. The rats were fed individually each day, receiving slightly more feed than it was expected they would consume. This helped to prevent wastage and rancidity. The rats were weighed each week.

The rats were housed in individual cages in a constant temperature room kept at 26 to 27 degrees Centigrade. The cages had wire floors, permitting the droppings to fall onto a pan. This pan was cleared of droppings every two days. All dishes were washed once a week, or oftener if thought desirable.

The rations were prepared in the manner described by Boutwell[1]. They consisted of 50 per cent skim milk powder, 20 per cent lactose (Merck U. S. P.), and 30 per cent fat. The resulting ration thus had a high lactose content--approximately 50 per cent. The skim milk powder was the sole source of the B-vitamins and protein. The milk powder was extracted 16 hours with anhydrous diethyl ether under vigorous agitation at room temperature. Toward the end of the period the solvent was changed after filtration at a water pump. The milk powder was mineralized so that there were 1.5 mg. of elemental iron and .15 mg. each of elemental copper and manganese per 10 grams of complete rations.

The two fats used in the rations were unsalted, sweet-cream butter fat (made from mixed herd milk obtained from the College Dairy Farm) and corn oil (Mazola oil). The fat-soluble vitamins were added to the fats before mixing with the skim milk powder and the lactose. The amounts of vitamins added per 100 grams of fat were: beta-carotene, 2 mg.; alpha-tocopherol, 8 mg.; calciferol, .05 mg.; and 2 methyl-1,4-naphthoquinone, .75 mg. Rations were prepared every two

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[1] Private Communication, September 28, 1944.

weeks or oftener and stored in the refrigerator in small containers. One batch of feed was prepared by Boutwell at Wisconsin and was used to feed rats of experiment IV-C.

Results and Discussion

The results obtained in the growth experiments are shown in Table 1. In all cases, the average weight gains of the rats fed on butter fat ration were greater than for the corn oil animals. In four of the seven cases, as will be discussed below, these differences were found to be significant. The rats on the butter fat ration also consumed more feed. The ratio of weight gain to feed consumption is similar for the corn oil and the butter fat fed rats, except in experiment II-B. In this short time experiment rats on butter fat appeared to utilize feed more efficiently.

Male rats which were fed for six weeks on the rations prepared in this laboratory (experiments II-A, IV-B, and V-A) showed very nearly the same ratio of weight gain to feed consumption between experiments and within experiments. The females, however, showed smaller ratios on both rations than in the case of males.

The greater weight gain and feed consumption for rations made in this laboratory over identical rations prepared by Boutwell, et. al., at the University of Wisconsin indicated that a difference existed between the rations since rats of

Table 1. The distribution of the rats, the weight gains, and food consumption in growth studies.

Experiment no.	Sex	Age at start	Feed	Number rats	Average weight at start	Average gain	Average difference in gains	Average food consumed	Average difference in feed consumption	Ratio of weight gain to feed consumption
		days			grams					
II-B	male	21	Corn oil	6	39.5	56.66*	89.34	110.08	2.72	0.514
			Butter fat	6	39.5	66.00*		112.80		0.584
IV-A	male	22	Corn oil	12	41.6	59.42*	11.75	121.13	22.91	0.491
			Butter fat		41.6	71.17*		144.04		0.494
II-A	male	21	Corn oil	6	39.7	158.83	4.84	366.79	9.12	0.433
			Butter fat	6	39.5	163.67		375.91		0.435
IV-B	male	22	Corn oil	6	41.3	153.17	14.33	350.38	33.20	0.437
			Butter fat	6	41.5	167.50		383.58		0.437
IV-C	male	22	Corn oil**	6	41.3	130.83	3.67	306.83	35.34	0.426
			Butter fat**	6	41.3	134.50		342.17		0.393
V-A	male	21	Corn oil	15***	39.7	121.27	21.73	300.27	31.60	0.404
			Butter fat	15***	40.1	143.00		331.87		0.431
V-B	female	21	Corn oil	11***	41.2	104.55	11.54	279.64	37.63	0.374
			Butter fat	11***	41.6	116.09		317.27		0.366

* after six weeks except experiments II-B (2½ weeks) and IV-A (3 weeks)
** Wisconsin feed
*** litter mates, raised in this laboratory; others from Sprague-Dawley

the same group were used. The only known differences were in the skim milk powder and the fats used. Since this happened on both corn oil and butter fat rations, it was possibly due to a difference in the skim milk powder. The ratios of weight gain to feed consumption for the Wisconsin feeds were also lower than those for the rations prepared in this laboratory.

It may be noted that the ratio was greatest for rats fed two and one-half and three weeks. It was during this period that the rats showed the greatest rate of growth.

The differences obtained in weight gains and in food consumption were statistified according to the t-test as described by Lindquist (22). It was found that, at the one per cent or the five per cent level of confidence, the weight gain differences were significant for experiments II-B, IV-A, V-A, and V-B. There were significant differences at the one per cent level between the Wisconsin ration and the ration produced in this laboratory. The differences in feed consumption for experiments IV-A, V-A, and V-B were significant at the one per cent level. The difference in feed consumption of the Wisconsin corn oil ration and that produced in this laboratory was significant at the one per cent level, but that for the butter fat rations was not. It was noted that the variance within groups was in most cases greater for rats fed on the butter fat ration than it was for rats fed on the corn oil ration.

In general all rats appeared normal except that some had bad diarrhea. At the start, the amount of diarrhea in the butter fat group was about the same as it was for the corn oil animals. This condition varied in severity between groups of animals and usually cleared up slower in the case of the corn oil animals than for the butter fat animals. A few animals showed slight lower abdominal alopecia. This was not at all consistent for one ration or from experiment to experiment. Since there was only one fatality (his litter mate was discarded also), it was felt that there was no serious lack of any nutrient in either ration. (This rat was in the butter fat group of experiment V-A. He appeared healthy the day before his death and at that time his weight was normal.)

MAZE LEARNING EXPERIMENTS

Procedure

Rats used in this study were all males obtained from Sprague-Dawley and were placed on this experiment following growth studies. The handling of these rats previous to the maze learning tests was described in the previous section. Rats of experiment I and III were raised by Boutwell, et. al., and sent to this laboratory by express following the growth studies in which they used rations prepared in the Wisconsin laboratory but of composition similar to those produced in this laboratory. The rats were in transit two days, received no feed during that time, and arrived in good condition. Some of the rations used in this experiment were made at the University of Wisconsin and the rest were made in this laboratory. In Table 2, these rations are called Wisconsin or Kansas rations, depending on the source.

Two or three days before maze learning was started, the amount of feed given was cut sharply to motivate the animals. When maze running was begun, the amount of feed was increased to around six grams, each rat being offered the same amount. Feeding was done at the same time each day. Water was given _ad libitum_. All feed was weighed out, and any remaining 30 minutes after feeding was removed. This method of restricted feeding seemed to keep both groups of rats well motivated and to about the same degree, yet it allowed for the slow growth of the rats from week to week after they once became adjusted to the restricted diet.

The mazes used for testing learning ability were an enclosed box maze and an elevated multiple-T maze. The floors of both mazes were movable and were changed after each rat had run thru themaze. This aired the maze and helped eliminate the possibility of a rat trailing another by scent. More complete descriptions and diagrams of the mazes are in the appendix. These mazes were essentially the same as those used by Alm and Whitnah (1).

The age at which the various rats were started on the mazes differed considerably (see Table 2). The rats were used first on the growth experiments, then were given maze learning tests. Since some of the growth experiments were continued for six weeks and others for only three weeks, the rats were not at the same age at the completion of the growth experiments. Also, there were small differences in the time required for the shipment of the rats.

The first two days the rats ran the box maze consisted of four group-orientation trials, two on each of the two days. The groups were composed of three rats. These trials seemed adequate for the animals to get used to the maze and to run rather freely. Small amounts of the experimental rations were used as bait. The following day individual trials were started. Exact records were kept of both the errors and the time required to run the maze. A sample of data collected during one day is in the appendix.

The box maze running order for experiment I animals was to run the corn oil animals all trials, then run the butter fat animals. After that an alternation technique was used so that every rat had one trial before any of the rats received a second trial. Also, the groups were reversed from day to day. One day the corn oil rats ran the first trials of the day; the next day the butter fat animals were put thru first. It was thought that this method would tend to equalize any differences that were produced by the order of running or

Table 2. Rats used in maze learning experiment.

Experiment	No. rats*	Rations	Where fed before maze running	Age in days at various stages: State of ad lib. feeding	State of restricted feeding	Start box maze	Start T maze	Finish
I	12	Wis.	Wis.	20	66	69	88	103
II-A	12	Kan.	Kan.	21	63	65	80	91
II-B	12	Kan.	Kan.	21	43	45	62	72
III	24	Wis.**	Wis.	21	43	46	61	71
IV-A	24	Kan.	Kan.	22	43	45	55	65
IV-B	12	Kan.	Kan.	22	64	67	77	88
IV-C	12	Wis.	Kan.	22	64	67	77	88

The rations are designated as Wis. or Kan., depending upon whether they were made at the University of Wisconsin or in this laboratory. Similar designations are made for the place at which the rats were fed before maze running.

* One-half of rats on each ration
** Kan. after 43 days of age

time of day. The rats within a group were run thru in random order. Five trials were given to each rat each day for eight days, except in experiments I, II-A, and II-B. Figure 1 shows graphically the average errors made when rats of experiment II-A were run a total of sixty trials on the box maze. The greatest improvement in average scores was observed during the first six or eight days, tending to level off after this period. On the basis of these results, which were similar in experiments I and II-B, it was decided to run the rats only forty trials on the box maze.

After the rats had received sufficient trials on the box maze, they were transferred to the T maze. Four preliminary group trials were given as for the box maze before individual trials were started. Alternation between groups was accomplished by running three corn oil rats and three butter fat rats in each group of six. This could be done very conveniently since there were six individual feed boxes at the end of the maze. Twenty-six trials were given each rat at the rate of three per day, ending with two on the final day of the experiment. Maze running on both mazes was started at approximately the same time each day. Figure 2 shows the average errors made per day by experiment II-A rats on the T maze, and in a general way it indicates the improvement observed from day to day by all groups.

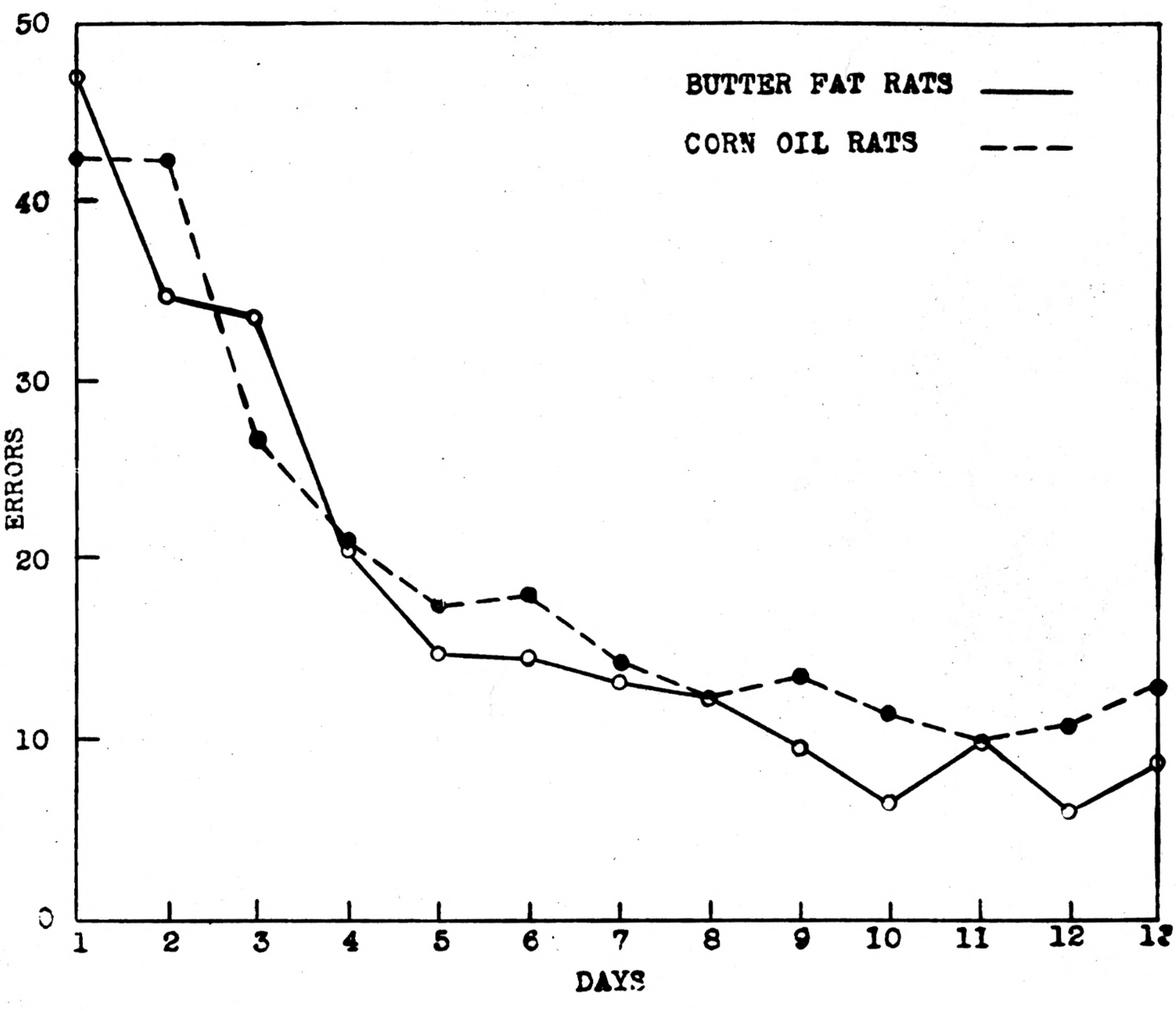

Fig. 1. Average errors per trial per day for experiment II-A on the box maze.

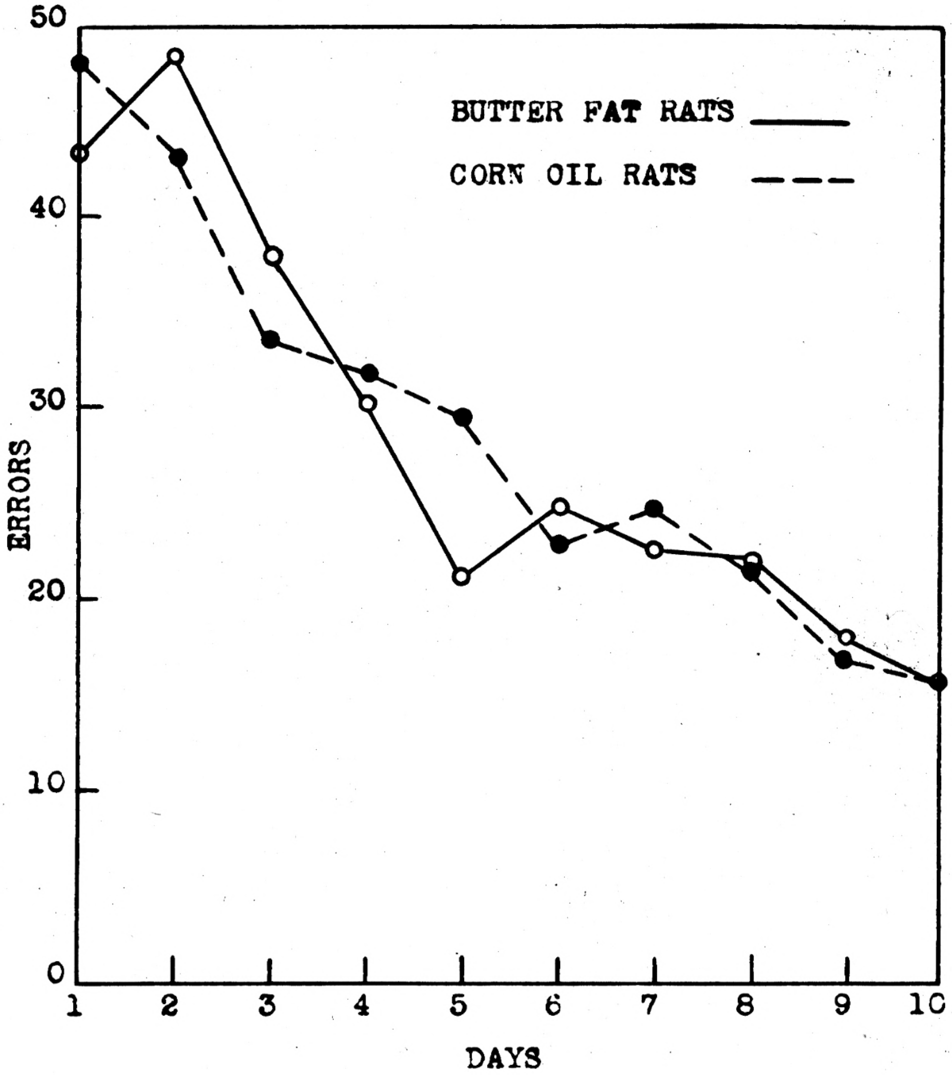

Fig. 2. Average errors per trial per day for experiment II-A on the T maze.

In all cases an attempt was made to keep the rats under identical conditions in so far as possible. Rats were transported to the maze laboratory in special cages built to protect the animals from the weather. Following maze running, the animals were returned to the constant temperature room and fed as previously described.

Motivation and Food Choice

In an effort to find out which ration the rats preferred, food choice trials were given some groups of rats after they had finished maze study. Twelve rats were used on each of the three experiments. These trials consisted of letting rats, one at a time, run down a short passageway which had two feed cups at the end. For experiment II-B rats, the feed pans for the two rations were just alike and were placed at the end of the passageway in random order and changed about during the tests. For experiment III the corn oil ration was placed in a black cup and the butter fat ration in a white cup. The two cups were in the same relative position each day. The color of thefeed cups for experiment IV-B was reversed. The butter fat ration was always to the left of the corn oil ration at the end of the passageway. This was intended to make it possible for the rats to learn the position of their favorite food, if they had such, so they could go directly to that ration when put in the passageway. The animals were kept on restricted feeding during this time so they would be motivated.

Daily records were kept, starting with the second day, of the feed each rat ate first and the one at which the rat ate at least 15 seconds. If the rat made no definite choice but kept alternating between the rations every few seconds, the first and third choices were recorded. The third choice or the ration at which the rat ate for 15 seconds are considered as the last choice in Table 3. Each rat was given two trials each day. Records were kept for four, six, and nine days for rats of experiments II-B, III, and IV-B respectively. It was believed this technique might give an indication of the food preference of the rats previously kept for seven to ten weeks on either the butter fat or the corn oil ration.

The results appearing in Table 3 indicate a slight tendency for rats in experiments II-B and III to prefer the ration they had been receiving previously. The experiment IV-B rats on butter fat ration showed a definite preference for the corn oil ration. These results are difficult to understand, but certainly nothing could be wrong with the butter fat ration since the rats which previously received the corn oil ration selected the butter fat ration almost as frequently as they did the corn oil ration. Also, another batch of butter fat ration was made up for use, but the butter fat rats still preferred the corn oil ration.

Table 3. Food choice of the rats fed butter fat and corn oil rations.

Experiment	Group	First choice		Last choice	
		Corn oil	Butter fat	Corn oil	Butter fat
II-B	Corn oil	26	22	28	20
	Butter fat	18	30	14	34
III	Corn oil	34	38	45	27
	Butter fat	15	57	18	54
IV-B	Corn oil	57	51	55	53
	Butter fat	75	33	83	25
Total	Corn oil	117	111	128	100
	Butter fat	108	120	115	113

A summary of these three experiments would suggest there was, on the whole, not enough difference in the ration preferences to cause difference in motivation in the growth or maze learning studies. Further evidence for the similarity of motivation on the two rations may be derived from the observations that the rats on both rations almost invariably began eating immediately upon entering the food box at the end of the maze trial.

In a study of food choice using an <u>ad libitum</u> feeding technique, Deuel and Movitt (6) found that rats ate more butter fat ration, or a vegetable fat ration flavored with diacetyl or commercial butter flavor, than they did unflavored vegetable oil rations. He believed this was due to the fact that rats preferred the butter flavor. In the present study it should be noted that no butter flavor of any kind was added to the corn oil rations. Though using a different, but probably no less valid, technique for investigating food preference, this series of experiments did not show the rats preferred a butter flavor. Further, the rats did not select one or the other food as more adequate, even though they had been on the experimental diets for seven to ten weeks.

Results and Discussion of the Maze Learning Experiments

It was thought desirable to attempt combining all experimental groups to get an over-all result. In order to be able to do this, it was necessary to show statistically that all groups of rats were random samples of the same population. This was accomplished by analysis of variance, as described by Lindquist (22). The variance within groups was compared with the variance between groups. By this treatment, four of the eight ratios of the variances were found to be statistically significant at the one per cent level of confidence and one at the five per cent level; therefore the hypothesis that the rats were random samples of the same population had to be rejected and all the groups could not be pooled. Table 4 is an example of one of the eight groups of calculations made.

Since there were age differences in the rats, this might have been the factor which prevented pooling of the results of the whole experiment. Accordingly, the hypothesis that rats which started on the maze at a younger age (i.e., experiments II-B, III, and IV-A) were random samples from the same population was set up. The analysis of variance for these experiments showed four significant ratios of variances, so they could not be pooled. Since the rats were the same age, had been obtained from litters at the same place, had been fed on the same ration, and had been handled in the same way, there must be unknown factors which produce the differences between groups.

Table 4. Analysis of variance for the butter fat rats for errors made on the box maze.

	Degrees of freedom	Sum of squares	Variance	Ratio(F)
Between groups	6	4,061.91	676,985.00	1.67188
Within groups	47	53,196.53	1,131,841.06	
Total	53	57,258.44		

The results obtained on each experiment in the maze learning studies were analyzed statistically and appear in Table 5. From these it can be seen that the butter fat rats averaged fewer errors and required less time in maze running than was the case with the rats fed on the corn oil rations. In some of the experiments the corn oil rats made better scores, as shown by the negative differences. To statisticize the results, the differences were divided by the standard error of the difference to get the significance ratio. If this value is above three, the chances are 99.9 in 100 that the true difference is greater than zero. For a significance ratio of two, the chances are about 98 in 100. The value of three usually must be exceeded in order to say that the difference is statistically significant. Using this measure of significance, the only differences that were significant were the time scores on the box maze for experiments II-B and IV-B. Of the 28 cases, two other differences (box maze errors for experiments II-B and IV-B) showed ratios above two and could be considered as slightly significant. In all cases the difference favored the rats fed the butter fat ration.

Since the number of animals in each group was rather small, it was thought advisable to use the t-test as described by Lindquist (22) for testing the significance. The t-test was designed by Fisher for use with small samples. The t-values were slightly lower than the significance ratios. Experiments II-B and IV-B show significant differences at the one per cent level in the case of the time on the box maze, and experiment II-B shows a significant difference for errors on the box maze at the five per cent level of confidence. The other t-values were too low to have any significance. From this it could be concluded that there was no consistent difference in the effect of the butter fat and the corn oil rations on the maze learning ability of the rats under the conditions of this experiment. The results indicate that variations in unknown factors entering into the different experiments may cause the inability to reproduce similar results.

These results do not rule out the possibility that the two rations may influence the maze learning ability of albino rats if the conditions are controlled so as to eliminate the differences in important unknown factors. Further investigation using larger groups of rats, if at all possible, is suggested as a result of this study. Before weaning, the rats received normal milk fat, and reserves of dietary factors which may be essential, though not present in corn oil, may have been accumulated sufficiently to meet the requirements for the period of this experiment. Work with younger rats will be necessary to find the answer to this question. Since medulation in rats is fairly well completed by the twentieth day, it would be expected that any differences in the effect of these rations on learning ability would be more pronounced if younger rats were used.

Table 5. Results of the maze learning experiments.

Experiment group	Average (per rat)	Difference	Significance ratio*	t-values **	Level of confidence of t-values %
Box maze - errors					
I					
Corn oil	157.17	31.34	1.97	1.793	20
Butter fat	125.83				
II-A					
Corn oil	137.00	5.67	0.63	0.571	-
Butter fat	131.33				
II-B					
Corn oil	161.00	28.83	2.44	2.230	5
Butter fat	132.17				
III					
Corn oil	125.25	-19.17	1.08	1.033	-
Butter fat	144.42				
IV-A					
Corn oil	143.87	- 4.38	0.20	0.191	-
Butter fat	148.25				
IV-B					
Corn oil	154.67	27.67	2.04	1.860	10
Butter fat	127.00				
IV-C					
Corn oil	145.33	8.33	0.52	0.475	-
Butter fat	137.00				
Box maze - time in seconds					
I					
Corn oil	402.17	42.67	1.11	1.010	-
Butter fat	359.50				
II-A					
Corn oil	349.25	4.67	0.16	0.146	-
Butter fat	344.58				
II-B					
Corn oil	474.00	148.83	3.60	3.290	1
Butter fat	325.17				
III					
Corn oil	263.17	-30.79	1.08	1.038	-
Butter fat	293.96				
IV-A					
Corn oil	306.79	-13.08	0.33	0.321	-
Butter fat	319.87				
IV-B					
Corn oil	563.00	182.75	3.99	3.646	1
Butter fat	380.25				
IV-C					
Corn oil	519.42	96.50	1.04	0.947	-
Butter fat	422.92				

Table 5 (concl.).

Experiment group	Average (per rat)	Difference	Significance ratio*	t-values **	Level of confidence of t-values %
T maze - errors					
I					
Corn oil	88.67	- 0.16	0.01	0.006	-
Butter fat	88.63				
II-A					
Corn oil	117.83	0.83	0.04	0.036	-
Butter fat	117.00				
II-B					
Corn oil	125.00	31.50	1.18	1.078	-
Butter fat	93.50				
III					
Corn oil	52.25	-15.33	1.30	1.247	30
Butter fat	67.58				
IV-A					
Corn oil	63.92				
Butter fat	57.33	6.59	0.74	0.706	-
IV-B					
Corn oil	97.50	7.33	0.27	0.248	-
Butter fat	90.17				
IV-C					
Corn oil	91.33	22.5	1.26	1.151	-
Butter fat	68.83				
T maze - time in seconds					
I					
Corn oil	952.00	-144.42	0.71	0.652	-
Butter fat	1096.42				
II-A					
Corn oil	1602.17	433.09	1.65	1.502	20
Butter fat	1169.08				
II-B					
Corn oil	1465.25	564.50	1.86	1.693	20
Butter fat	900.75				
III					
Corn oil	496.88	- 76.12	1.23	1.177	-
Butter fat	573.00				
IV-A					
Corn oil	683.50	104.46	1.31	1.259	30
Butter fat	579.04				
IV-B					
Corn oil	1042.50	58.17	0.31	0.286	-
Butter fat	984.33				
IV-C					
Corn oil	995.42	55.42	0.40	0.363	-
Butter fat	940.00				

* Significance Ratio = $\frac{M_1 - M_2}{\sigma_{(M_1 - M_2)}}$; M_1 and M_2 are the means of the two groups, and $\sigma_{(M_1 - M_2)}$ is the standard error of the difference of the means (21).

** $t = \frac{M_1 - M_2}{\sqrt{\left(\frac{\Sigma d_1^2 + \Sigma d_2^2}{n_1 + n_2 - 2}\right)\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$; M is the mean, d is the deviation from the mean, and n is the number of cases in the groups, here called 1 and 2 (22).

SUMMARY

1. The average weight gains were greater in all cases for rats fed on the butter fat ration of high-lactose content than on the corn oil ration. In four of the seven experiments the difference in weight grains was statistically significant at the five per cent level or better.

2. The average food consumption was greater for the animals fed on the butter fat ration than on the corn oil ration. Three of these differences were significant at the five per cent level or better.

3. The ratio of weight gain to feed consumption was very nearly the same for the rats on the butter fat ration and the corn oil ration. This ratio was slightly lower for females than it was for male rats.

4. There were significant differences in the weight gains produced by the Wisconsin rations and those produced in this laboratory; therefore a difference in these feeds must exist.

5. The rats as a whole did not show enough preference for one ration over another to produce any difference in motivation between the groups.

6. There was no consistent difference in the effect of the butter fat and the corn oil rations on the maze learning ability of albino rats under the conditions of this

experiment.

7. Results in the maze learning experiments could not be reproduced consistently due to differences in unknown factors.

8. In future studies, the rats should be started on the experiments at a younger age, and the differences in the unknown factors should be identified and controlled.

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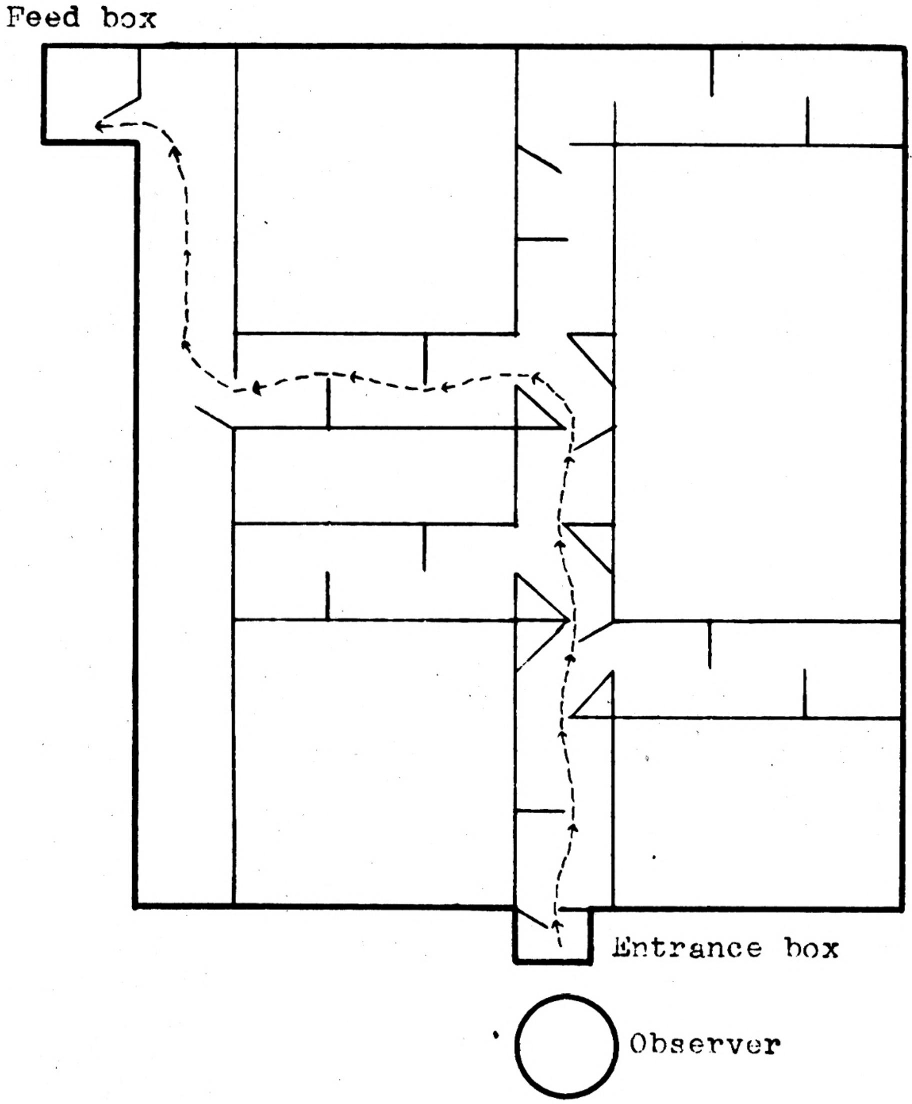
APPENDIX

Description of the Box Maze

The enclosed box maze consisted of alleys enclosed by eight inch wooden sideboards, a wire top to keep the rats from climbing out, and a movable black oil cloth floor. The maze was made so that it could be lifted off the floor for aeration and the rolling of the floor to a fresh part of the oil cloth. This method of airing the maze and changing the floor tended to prevent a rat from tracing another thru the maze. The maze was lifted and the floor rolled after each rat ran the maze.

The maze was well lighted from above, and the animals were observed in an overhanging mirror. Black drapes completely enclosed the maze. The retracing of alleys was prevented by the quiet closing of doors after the rat had moved on a short distance. The maze was essentially the same as that used by Alm and Whitnah (1).

Floor plan of box maze



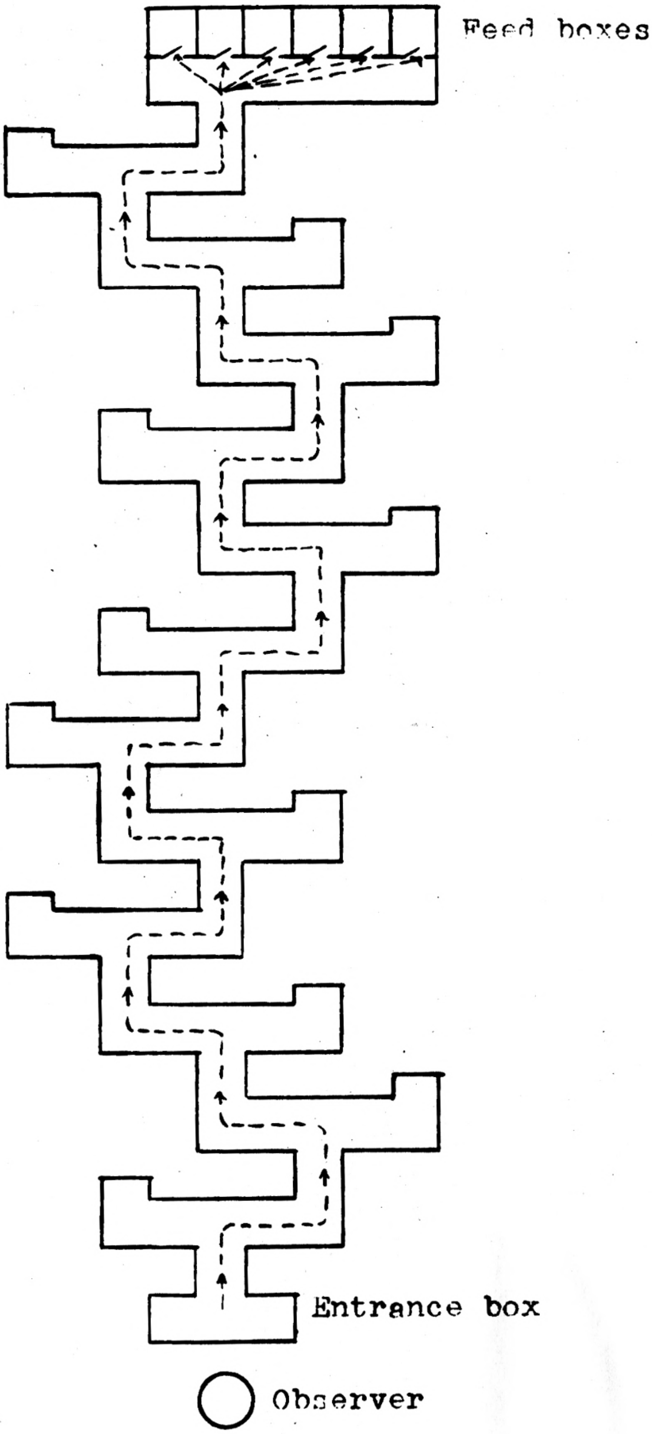
Description of the T Maze

The elevated T maze was composed of 12 T-shaped alleys with variations of right and left turns. The alleys were three inches wide and had low metal sides. The bars of the T's were covered with black oil cloth strips which could be changed by the rotation of a roller. This was done after six rats had run thru the maze. Retracing was largely eliminated by dropping the hinged stems of the T's behind the animals.

Signboards of sandpaper and white paper were used to aid the rat. Sandpaper designated right turns and was placed in the floor of the stem of the T's. Left turns were designated by the presence of white paper hung from the walls in front of the stem. At the end of the maze the rats entered individual feed boxes. The door then could be closed and all feed boxes filled with animals before it was necessary to remove the animals to make room for others. In each group of six rats put thru the maze, there were three corn oil rats and three butter fat rats.

This was the same maze which Alm and Whitnah (1) used.

Floor plan of T maze



Data Sheet Used for the Maze Learning Experiments

Experiment No. IV B

Date April 9 Observer R. Shimer

Maze Box

Rat No.	Trial 31		Trial 32		Trial 33		Trial 34		Trial 35	
	Err.	T.	Err.	T.	Err.	T.	Err.	T.	Err.	T.
25	3	16½	2	6	3	6	2	6	2	4½
26	5	25	2	8½	2	7½	2	6½	3	11
27	0	11½	4	17	0	* 20	9	27	3	16
28	0	4	2	4	0	2½	2	5	0	2½
29	2	8	3	5	2	7	2	6½	3	6
30	3	9½	2	10½	3	8½	2	7½	0	5
37	2	5	3	4½	2	7	2	5	2	4½
38	2	6	2	5	2	4½	2	4	3	5½
39	2	4	3	4	3	3½	2	3½	2	4
40	1	5	3	4½	2	4	2	4	1	5
41	2	7	2	4½	2	4	2	4½	2	4½
42	3	9	2	5½	7	26	2	5	1	5

Err.--Errors T.--Time in Seconds

Remarks:

* Seemed disturbed