ments similar to Experiment 1, we have found that 3 $\gamma$ of folic acid given daily between the 35th and 49th days will allow a change in growth from about 93 grams to 140 grams, an increase in white cell count from about 4,000 to 10,000, and a change in the granulocyte count from about 300 to 1,000.

P-aminobenzoic acid has been previously tried for its effect on the syndrome produced with sulfasuxidine in rats fed a purified diet, but with little success. However, it was included in Experiment 3 to determine whether it might allow some subtle effect of thymine to be shown. This was not the case under our conditions.

### Results and Discussion

Our data indicate that thymine has no curative or preventive effect on the course of folic acid deficiency in the rat with respect to either growth or any part of the blood picture, when it is fed daily in amounts equivalent to 2.1, 3.5, 4.2, and 7.2 $\gamma$ of folic acid as determined microbiologically in our laboratory.[3]

In view of the striking observations reported by Spies, *et al.* in human patients (*5*), these results on rats are interesting. They point to the fact that thymine is not a true substitute for folic acid and indicate that when folic acid-like activity is shown by thymine, pyrimidines, or other preparations, some indirect mode of action must be operating. Such a viewpoint is in harmony with the fact that thymine is chemically very unlike folic acid.

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## Experimental Diabetes and Diet


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The influence of different diets on sensitiveness to alloxan and experimental pancreatic diabetes in the white rat has been investigated.

Two hundred twenty-three female rats weighing between 90 and 120 grams were divided into several groups and fed different diets for a period of one month. At the end of the month and 12 hours after the last meal they were injected intraperitoneally with 160 mg./kg. of alloxan. The mortality rate was considered during one week after the injection, and in all cases high hyperglycemia preceded death.

[3] The thymine used in this work was chemically pure and found to have 280 $\gamma$ of folic acid activity/gram.

The results were as follows (see Table 1):

(1) In the group given a high carbohydrate diet the mortality due to alloxan was 40 per cent.

(2) In the group given a low protein diet (10 per cent casein content) there was 90 per cent mortality. Increasing the protein content from 20 to 45 per cent increased the resistance to alloxan (40 and 33 per cent mortality).

TABLE 1

| Diets | Wheat flour (%) | Corn flour (%) | Casein (%) | Fat (%) | Yeast (%) | Cod liver oil (%) | Salt mixture (McCollum Davis) (%) | No. of rats used | Mortality (%) |
|---|---|---|---|---|---|---|---|---|---|
| (a) High carbohydrate | 35 | 34 | 20 | 0 | 5 | 5 | 1 | 15 | 40 |
| (b) High protein | 10 | 34 | 45 | 0 | 5 | 5 | 1 | 15 | 33 |
| (c) Low protein | 40 | 39 | 10 | 0 | 5 | 5 | 1 | 15 | 90 |
| (d) High lard | 10 | 25 | 20 | 34 | 5 | 5 | 1 | 20 | 100 |
| (e) High lard and high protein | 14 | 15 | 30 | 30 | 5 | 5 | 1 | 15 | 100 |
| (f) High ox fat | 10 | 25 | 20 | 34 | 5 | 5 | 1 | 14 | 86 |
| (g) High lard, and methionine (200 mg./kg./day) | 10 | 25 | 20 | 34 | 5 | 5 | 1 | 10 | 30 |
| (h) High lard, and sulfanilamide (250 mg./kg./day) | 10 | 25 | 20 | 34 | 5 | 5 | 1 | 10 | 90 |
| (i) High lard, and choline (300 mg /kg./day) | 10 | 25 | 20 | 34 | 5 | 5 | 1 | 15 | 100 |
| (j) High lard, and thiouracil (200 mg./kg./day) | 10 | 25 | 20 | 34 | 5 | 5 | 1 | 10 | 10 |
| (k) High olive oil | 10 | 25 | 20 | 34 | 5 | 5 | 1 | 15 | 40 |
| (l) High butter | 10 | 25 | 20 | 34 | 5 | 5 | 1 | 15 | 33 |
| (m) High oleomargarine | 10 | 25 | 20 | 34 | 5 | 5 | 1 | 15 | 21 |
| (n) High corn oil | 10 | 25 | 20 | 34 | 5 | 5 | 1 | 15 | 13 |
| (o) High coconut oil | 10 | 25 | 20 | 34 | 5 | 5 | 1 | 14 | 0 |
| (p) High lard (30%) and coconut oil (10%) | 9 | 20 | 20 | 40 | 5 | 5 | 1 | 10 | 20 |

(3) High lard and high ox fat diets decreased strongly the resistance to alloxan (100 and 86 per cent mortality, respectively). This effect was not modified in the former either by increasing the casein content from 20 to 30 per cent (100 per cent mortality) or by treatment with choline or sulfanilamide. In contrast, the treatment with methionine or thiouracil corrected the hypersensitiveness (30 and 10 per cent mortality, respectively).

(4) The sensitiveness to alloxan was not modified when high olive oil or high butter diets were administered (40 and 33 per cent mortality, respectively), but there was an increased resistance to alloxan in the rats fed either a high oleomargarine diet (21 per cent mortality) or in those fed a high corn oil diet (13 per cent mortality).

(5) Complete protection to alloxan in rats fed a high coconut oil diet (0 per cent mortality) was observed.

(6) In rats fed a high lard diet modified by the addition of 10 per cent coconut oil, a partial protection to alloxan was observed (20 per cent mortality).

The role of the quality of diets and the amount of food fed on the initiation and evolution of experimental pancreatic diabetes in rats has also been studied.

Fifty-one male rats weighing about 130 grams were subtotally pancreatectomized (95 per cent of the pancreas) and

divided into three groups, each of which was fed one of the diets shown in Table 1 (a, b, and d).

As shown in Table 2, the high protein diet showed a protective effect and the high lard diet an unfavorable effect in comparison with that which was high in carbohydrate.

TABLE 2

| Time after operation (mos.) | High protein diet | | High carbohydrate diet | | High fat diet | |
|---|---|---|---|---|---|---|
| | Diabetes | | Diabetes | | Diabetes | |
| | Total | % | Total | % | Total | % |
| 1 | 4/16 | 25 | 2/18 | 11.1 | 7/17 | 41.1 |
| 2 | 9/16 | 56.2 | 14/18 | 77.7 | 17/17 | 100 |
| 4 | 10/16 | 62.5 | 15/17 | 88.2 | 15/15 | 100 |
| 5 | 10/16 | 62.5 | 12/13 | 92.3 | 15/15 | 100 |
| 6 | 11/15 | 73.3 | 11/12 | 91.6 | 14/14 | 100 |
| 7 | 11/15 | 73.3 | 11/11 | 100 | 10/10 | 100 |
| 8 | 9/12 | 75.0 | 10/10 | 100 | 5/5 | 100 |

In overfed rats (fed three times daily with a high carbohydrate diet) the evolution of diabetes was much faster than in those fed once a day and eating less food. Furthermore, the same diet but restricted in quantity (about 80 per cent of the amount of food eaten by a normal rat) produced a marked delay in the time of initiation of diabetes (see Table 3).

TABLE 3

| Time after operation (mos.) | Underfed | | Overfed | | Ad libitum | |
|---|---|---|---|---|---|---|
| | Diabetes | | Diabetes | | Diabetes | |
| | Total | % | Total | % | Total | % |
| 1 | 1/13 | 8 | 4/17 | 23.5 | 2/18 | 11.1 |
| 2 | 5/12 | 41.6 | 15/15 | 100 | 14/18 | 77.7 |
| 4 | 3/7 | 42.8 | 10/10 | 100 | 15/17 | 88.2 |
| 5 | 1/7 | 14.3 | 7/7 | 100 | 12/13 | 92.3 |
| 6 | 4/7 | 57.1 | 5/5 | 100 | 11/12 | 91.6 |
| 7 | 3/7 | 42.8 | 3/3 | 100 | 11/11 | 100 |
| 8 | 3/7 | 42.8 | 3/3 | 100 | 10/10 | 100 |

Diabetes appeared first in those rats which received their food once a day (in a quantity proportional to their body weight) rather than in those receiving the same amount of food divided into three feedings at 7-hour intervals. It may be supposed that the higher postprandial hyperglycemia of the former diet exerts an unfavorable effect, whereas the postprandial hyperglycemia resulting from the latter is less intense.


SUMMARY

In the experiments described the following observations have been made:

(1) The toxic and diabetogenic action of alloxan increases in rats fed a low protein diet and even more so in the case of a high lard or ox fat diet.

(2) The action of these fat diets was corrected by the addition of methionine, thiouracil, or coconut oil, but there was no modification either by addition of choline or sulfonamide or by increase in the protein proportion.

(3) In rats fed other high fat diets (olive oil, butter) the actions of alloxan were not modified, but there was a slight diminution when high oleomargarine or corn oil diets were fed. However, complete protection was observed when a high coconut oil diet was administered.

(4) The unfavorable effect of the high lard diet was observed also on the initiation and evolution of diabetes due to subtotal pancreatectomy. Contrarily, feeding a high protein diet and especially treatment with thiouracil had a favorable effect.

(5) Diabetes appeared first in subtotal pancreatectomized rats which were overfed, then in those which ate ad libitum, and finally in those which were underfed. Diabetes appeared in rats fed a single meal before it did in those eating the same amount of food divided into three meals.


## Mechanism of the Antibiotic Action of Certain Quinones


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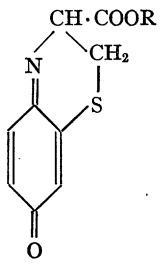
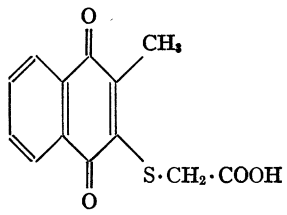
A widely accepted theory suggests that many antibiotics function chiefly because of their ability to interfere with the sulfhydryl groups of enzymes concerned in bacterial metabolism (*2*).

In a recently published report by Colwell and McCall (*3*) it is claimed that the inhibition of bacterial and fungus growth by 2-methyl-1,4-naphthoquinone and related quinones also is due to a reaction of the quinones with sulfhydryl compounds. This theory is supported by the fact that excess amounts of sulfhydryl compounds are able to suppress the antibiotic properties of 2-methyl-1,4-naphthoquinone. Kuhn and Beinert (*8*) have shown that p-benzoquinone and cysteine-ethylester-hydrochloride react by addition of the —SH compound to a free position in the quinone molecule; in two further steps a condensation product (I) is formed. Fieser and Fieser (*4*) describe the reaction between 2-methyl-1,4-naphthoquinone and thioglycolic acid, where an addition product of the formula (II) is formed.




(I)




(II)

As far as quinones are concerned, the theory described is, however, not a satisfactory one. As Colwell and McCall (*3*)