THE EFFECTS ON RATS OF CHRONIC EXPOSURE TO 34°C

I. THE EFFECT OF VARIATIONS IN THE DIET ON GROWTH AND ON THE ABILITY OF MOTHERS TO REAR PUPS TO WEANING AGE

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Summary

When supplements of Pentavite and a Vetemul–vitamin E mixture were added to stock diets, rats kept at room temperature were unaffected, but these supplements improved the growth rate of rats kept at 34°C and enabled some of the mothers to rear pups to weaning age.

When rats kept at 27°C were fed purified diets, they grew slowly and many mothers lost their litters between birth and weaning. Rats kept at 34°C also grew slowly on purified diets and no mother was able to rear pups to weaning age. Addition of liver to the diet improved growth and the ability to rear young in both groups, though litter survivals were still poor for those at 34°C.

For rats kept at 34°C, results for supplemented stock diets and for purified diets supplemented with liver were similar. These responses, however, were significantly below those for rats kept at room temperature and at 27°C. It is suggested that the differences between the animals at 34°C on these two diets and those kept at more moderate temperatures were due largely to the direct effects of heat exposure.

I. Introduction

Evidence from physical performance, from levels of storage and excretion, and from the appearance of classical signs of vitamin deficiency suggests that requirements of man and rats for a number of dietary factors are changed by exposure to high environmental temperatures (Mitchell and Edman 1951). Exposure to heat is also known to affect growth rate and reproductive potential of rats (Sundstroem 1930), but the possibility that these differences are due, partly or wholly, to increases in dietary requirements has received very little attention. Mills (1942, 1943*b*) was able to improve the growth rate of rats kept at 32·2°C by increasing the amount of thiamine and choline in the diet, and Macfarlane, Pennycuik, and Thrift (1957) found that though stock diets gave adequate growth and reproductive performance at room temperature, supplements of protein, vitamin A, B vitamins, and vitamin E effectively reduced the rate of resorption of foetuses in animals exposed acutely to 32–35°C.

The object of the present experiments on the influence of ambient temperature on rats was to find a diet which reduced the differences of nutritional origin as far as possible. In the first series of experiments two stock diets were tried. The effects of supplements were then investigated. In a second series of experiments, a purified diet was used. This was then supplemented with wheat and liver. Growth rate, the

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number of corpora lutea at 16 days gestation, and the number of pups surviving until weaning were used as criteria in judging the adequacy of the diet. The experiments were *not* designed to investigate the minimum requirements of any specific dietary factor for rats maintained at high environmental temperature.

TABLE 1
COMPOSITION OF STOCK DIETS

	Barastoc Dog Cubes	Red Comb Rat Mash
Moisture	9·7%	10·05%
Crude fibre	2·6%	—
Crude fat	5·6%	2·0%
Crude protein	19·1%*	15·6%†
Total minerals	6·1%	—
Calcium	1·05%	—
Phosphorus	1·0%	—
Sodium chloride	0·76%	0·5%
Magnesium	76 p.p.m.	—
Zinc	47 p.p.m.	—
Copper	13 p.p.m.	—
Iron	334 p.p.m.	—
Cobalt	0·15 p.p.m.	—
Vitamins		
Vitamin A	880 i.u./100g	330 i.u./100g
Carotene	99 i.u./100g	—
Thiamine	0·66 mg/100g	0·61 mg/100g
Riboflavin	0·55 mg/100g	0·17 mg/100g
Nicotinic acid	34 mg/100g	34 mg/100g
Choline	0·29 mg/100g	—
Vitamin D	110 mg/100g	55 i.u./100g
Total tocopherol	4·95 mg/100g	—
α-Tocopherol	2·31 mg/100g	—

* Protein sources: 71% grains, i.e. hulled oats, pollard, biscuit meal, toasted wheat flakes; 18% meat meal; 4% liver meal; 7% milk and whey products.

† Protein sources: maize meal 41%; bran 15%; pollard 27%; wheat meal 17%.

II. MATERIAL AND METHODS

(a) *Experimental Animals*

The experimental animals used were a strain of Wistar albino rats which had been bred in this Laboratory since 1940.

(b) *Temperature Control of Experiments*

In the first series of experiments, the rats were kept in cages in an incubator running at 34 (±1)°C. Humidity was not controlled, and on warm wet days relative humidity rose as high as 55%. Air movement round the cages was of the order of

TABLE 2

DAILY AMOUNTS PER RAT OF VITAMINS INGESTED AT 20 WEEKS OF AGE BY ANIMALS REARED AT ROOM TEMPERATURE AND AT 34°C AND FED STANDARD AND VITAMIN-SUPPLEMENTED DIETS

Vitamin	Red Comb Rat Mash	Mash plus Pentavite	Mash plus Pentavite, Vetemul–vitamin E, and Choline	Barastoc Dog Cubes	Cubes plus Pentavite	Cubes plus Pentavite and Vetemul–vitamin E
			Rats reared at room temperature			
Vitamin A	44 i.u.	131 i.u.	1560 i.u.	116 i.u.	203 i.u.	1632 i.u.
Thiamine	80 μg	106 μg	106 μg	86 μg	112 μg	112 μg
Riboflavin	22 μg	54 μg	54 μg	73 μg	104 μg	104 μg
Nicotinic acid	4·5 mg	4·7 mg	4·7 mg	4·5 mg	4·7 mg	4·7 mg
Pyridoxine	?	2·9 μg+?	2·9 μg+?	?	2·9 μg+?	2·9 μg+?
Pantothenic acid	?	7·1 μg+?	7·1 μg+?	?	7·1 μg+?	7·1 μg+?
Choline	?	?	107·1 μg+?	38·6 μg	38·6 μg	38·6 μg
Vitamin C	?	1·4 mg+?	1·4 mg+?	?	1·4 mg+?	1·4 mg+?
Vitamin D	7·3 i.u.	15·8 i.u.	158·6 i.u.	14·5 i.u.	23·1 i.u.	165·9 i.u.
Vitamin E	?	?	0·28 mg+?	0·30 mg	0·30 mg	0·59 mg
			Rats reared at 34°C			
Vitamin A	24 i.u.	111 i.u.	1540 i.u.	64 i.u.	151 i.u.	1580 i.u.
Thiamine	44 μg	70 μg	70 μg	48 μg	74 μg	74 μg
Riboflavin	12 μg	44 μg	44 μg	40 μg	72 μg	72 μg
Nicotinic acid	2·5 mg	2·7 mg	2·7 mg	2·5 mg	2·7 mg	2·7 mg
Pyridoxine	?	2·9 μg+?	2·9 μg+?	?	2·9 μg+?	2·9 μg+?
Pantothenic acid	?	7·1 μg+?	7·1 μg+?	?	7·1 μg+?	7·1 μg+?
Choline	?	?	107·1 μg+?	21·4 μg	21·4 μg	21·4 μg
Vitamin C	?	1·4 mg+?	1·4 mg+?	?	1·4 mg+?	1·4 mg+?
Vitamin D	4·0 i.u.	12·6 i.u.	155·4 i.u.	8·0 i.u.	16·6 i.u.	159·5 i.u.
Vitamin E	?	?	0·28 mg+?	0·17 mg	0·17 mg	0·45 mg

25 ft/min. Control animals were kept in cages at room temperature (which ranged from 21 to 35°C in summer and from 12 to 25°C in winter).

In the second series of experiments, two incubators and a cool room were used. These were kept at 34 (±1), 27 (±1), and 21 (±1)°C. Humidity was not controlled and air movement was of the order of 25 ft/min.

Because of the lack of humidity control, the effective temperature in all chambers used varied with diurnal and seasonal fluctuations in humidity. Moreover, the relative humidity in the chamber running at 34°C was always lower than that of chambers running at lower temperatures. Heat loss by evaporation at 34°C was therefore facilitated and the stress imposed by the high temperature was reduced to some extent.

III. Diets and Experimental Procedures

In the first series of experiments, the basic diets fed to the rats were two used routinely in this Laboratory. The information available from the manufacturer on the composition of these diets is shown in Table 1.

Growth rate and the survival of young were measured in rats kept at room temperature and at 34°C. Supplements were then added and the same measurements were repeated. The supplements used were: Pentavite (Nicholas Pty. Ltd.), a mixture of Vetemul (Nicholas Pty. Ltd.) and α-tocopherol acetate (Roche), and choline chloride (20% solution).

In early experiments Pentavite was administered in the drinking water, but in later experiments it was given on the food three times a week. In both cases the dose was 0·1 ml/rat/week. The choline chloride solution was also given with the food three times a week. The dosage was 0·40 ml/rat/week. The Vetemul–vitamin E mixture was given once a week at the rate of 2 ml/rat/week. By measuring the food intake of animals of 20 weeks of age at room temperature and at 34°C (viz. 13·2 g/rat/day for animals at room temperature and 7·3 g/rat/day for animals at 34°C) it was possible to calculate the vitamin intakes of these animals at the two different temperatures. These are summarized in Table 2.

Comparison of these data with published minimum requirements (Griffith and Farris 1942; Russell 1948; Brown and Sturtevant 1949; Albritton 1954; Cuthbertson 1957) suggests that intakes of some vitamins were inadequate for rats kept both at room temperature and at 34°C. Moreover, because the rats at 34°C ate less than those at room temperature, the position was more acute in this group. Intakes of amino acids and salts were similarly inadequate.

In the second series of experiments attempts were made to provide adequate and equal supplies of vitamins, amino acids, and salts for both control rats and for those kept at 34°C. This was achieved by preparing a low-calorie diet containing the correct proportions of vitamins, salts, and amino acids. The quantities of food eaten at the experimental and control temperatures were measured, and the results converted to calories. By subtracting the caloric value of the daily requirements of the basic diet from these values it was possible to calculate the amounts of sucrose which would have to be added to this diet in order to equalize the protein, salt, and vitamin intakes of the animals at these two temperatures.

TABLE 3

RESPONSES OF RATS REARED AT ROOM TEMPERATURE TO STOCK DIETS AND SUPPLEMENTED STOCK DIETS

Diet	No. of Rats	Weight at 20 Weeks (g) (±S.E.)	No. of Rats	Duration of Gestation (days) (±S.E.)	No. of Rats	No. of Corpora Lutea (±S.E.)	No. of Rats	No. of Litters Surviving at End of Lactation	No. of Pups Viable at 21 Days (±S.E.)
Mash	17	147±2·90	—	—	—	—	—	—	—
Cubes	11	154±3·60	11	22·1±0·16	11	10·18±0·40	11	10	7·64±0·61
Cubes, Pentavite	11	141±3·60	8	22·1±0·19	8	9·50±0·47	7	7	5·43±0·77
Mash, Pentavite, Vetemul–vitamin E, choline	12	139±3·45	10	22·3±0·17	10	9·10±0·42	10	10	8·10±0·64
Cubes, mash, Pentavite, Vetemul–vitamin E	13	156±3·31	12	22·0±0·15	12	8·92±0·39	11	11	7·00±0·61
Cubes, Pentavite, Vetemul–vitamin E	20	142±2·67	13	22·2±0·15	13	8·77±0·37	12	12	6·42±0·58

Three basic diets were used, differing only in their protein sources. In the first, casein was the only protein used, the second contained liver as well as casein, and the third contained wheat, liver, and casein. The casein used was lactic casein, the liver meal was prepared by drying and powdering fresh beef liver, and the wheat was whole crushed grain. The total amounts of each protein source added to the diets were determined by the limiting amino acid.

The salt mixture and the vitamin supplements used were the same in all three basic diets. The salt mixture was one based on that of Hubbell, Mendel, and Wakeman (1937). Salts formed 5% of the total diet. Most of the vitamins were given in the form of a powdered mixture made up in the proportions suggested by Cuthbertson (1957). Composition of 100 g of this mixture was as follows: thiamine 158 mg, riboflavin 596 mg, pyridoxine 158 mg, calcium pantothenate 948 mg, nicotinic acid 792 mg, folic acid 156 mg, vitamin B_{12} 2·4 mg, biotin 30 mg, menadione 78 mg, *p*-aminobenzoic acid 12·493 g, inositol 234 mg, filler, which included fillers present in proprietary tablets and powdered sucrose, 84·5 g. Vitamin E and essential fatty acids were supplied as a solution of 0·4 g α-tocopherol acetate in 100 ml sunflower seed oil. Vitamins A and D were supplied as Vetemul and choline was given as a 10% aqueous choline chloride solution. The amounts of powdered vitamin mixture, sunflower seed oil, and Vetemul added to each diet were based on Cuthbertson's (1957) estimate of vitamin requirements of rats kept at room temperature. Choline quantities, however, were increased to allow for the known increase in requirements at high environmental temperatures (Mills 1942).

When the rats were 20 weeks of age, the daily intake of vitamins and salts (g/rat/day) for all diets at all temperatures were: salt mixture 0·4, sunflower seed oil 0·25, vitamin mixture 0·0166, Vetemul 0·08, choline chloride solution 0·4.

The daily intake of the various protein sources (g/rat/day) in the three basic diets was:

	Casein Mixture	Liver–Casein Mixture	Wheat–Liver–Casein Mixture
Casein	2·9	2·3	2·0
Liver	—	0·5	0·5
Wheat	—	—	3·0

The daily intake of sucrose (g/rat/day) at the three different temperatures on the three basic diets was:

	Casein Mixture	Liver–Casein Mixture	Wheat–Liver–Casein Mixture
21°C	—	—	2·4
27°C	3·0	4·0	0·6
34°C	1·5	1·8	0

The rats used as controls in this series of experiments were kept at 27°C, since it was thought that the fluctuating environment at room temperature may have affected the results in the earlier series. A few measurements were also made of rats reared at 21°C on the wheat–liver–casein diet.

For each diet tried (e.g. for the three temperature groups given the wheat–liver–casein diet in the second series of experiments), the split-litter technique was

TABLE 4

POOLED RESULTS FOR RATS REARED AT ROOM TEMPERATURE AND RESULTS FOR RATS REARED AT 34°C AND FED STOCK AND SUPPLEMENTED STOCK DIETS

Diet	No. of Rats	Weight at 20 Weeks (g) (±S.E.)	No. of Rats	Duration of Gestation (days) (±S.E.)	No. of Rats	No. of Corpora Lutea (±S.E.)	No. of Rats	No. of Litters Surviving to 21 Days of Age	No. of Viable Young at 21 Days (±S.E.)
Rats reared at room temperature	96	147±1·21	54	22·2±0·07	54	9·26±0·18	51	50	7·00±0·28
Rats reared at 34°C									
Mash	15	119±2·46	5	24·6±0·80	10	7·40±0·62	10	0	0
Cubes	15	100*	5	24·8±0·18	6	8·17±0·81	6	0	0
Mash, Pentavite	10	108±3·01	—	—	—	—	—	—	—
Cubes, Pentavite (group A)	14	110*	10	23·5±0·25	10	7·30±0·62	10	1†	0·3‡
Mash, Pentavite, Vetemul–vitamin E, choline (group B)	13	132±2·64	11	23·6±0·18	11	7·73±0·60	11	3†	0·5‡
Cubes, mash, Pentavite, Vetemul–vitamin E (group C)	12	128±2·75	12	23·3±0·38	12	8·25±0·57	5	2†	1·0‡

* Weighed as a group.

† Significance of differences for survival of litters to 21 days of age for three groups of rats fed diets indicated are: Groups A and B, $\chi^2 = 0{\cdot}203$, $0{\cdot}7 > P > 0{\cdot}5$; groups A and C, $\chi^2 = 0{\cdot}675$, $0{\cdot}5 > P > 0{\cdot}3$; groups B and C, $\chi^2 = 0{\cdot}0053$, $0{\cdot}95 > P > 0{\cdot}90$.

‡ The values for these groups showed a skew distribution due to the fact that a number of mothers lost entire litters. Because of this standard errors had no meaning and have not been quoted.

employed. The animals used were female rats born to mothers reared at room temperature. At weaning, the pups were divided into two or three groups (depending on the number of experimental temperatures to be used) of 10–12 animals and placed in the incubators. From this time onwards they were fed only the diets to be investigated, and at 20 weeks of age they were weighed.

In the first series of experiments the animals were mated at ages between 20 and 24 weeks. Those kept at 34°C were removed from the incubator overnight and introduced into a cage at room temperature with a male which had been reared at room temperature. The following morning the females were returned to the incubator. The day on which sperm were found in the vaginal smear was counted as day 0 of the pregnancy. Laparotomy was performed under ether anaethesia on the sixteenth day of pregnancy, and corpora lutea, implants, and living foetuses were counted at this time. The numbers of corpora lutea gave a measure of the numbers of eggs released, although Brambell (1948) has some objections to the accuracy of the method. Pregnant females were removed to individual cages at room temperature on the twentieth day of pregnancy and were allowed to litter at that temperature. Nursing boxes were examined for new-born pups in the mornings and evenings only. Estimates of the duration of pregnancy were therefore not completely accurate, since neither the hour at which mating took place nor the hour of birth was known exactly. Counts of young born alive could be incomplete since mothers could have eaten some of the pups before the litters were found. When the pups were 4 or 5 days old, mothers of the groups which had been reared at 34°C and their litters were returned to the incubator running at that temperature.

In the second series of experiments, the animals were mated at the experimental temperatures to males reared at the same temperature. (There was evidence that these were no less fertile than those reared at room temperature.) Otherwise the handling of the animals was the same as that in the first series of experiments.

IV. Results

(*a*) *First Series of Experiments*

Results for rats reared at room temperature are shown in Table 3. Weights of rats at 20 weeks of age were variable, but it was not possible to relate variations to the diets fed. There was some evidence that these differences might be due to seasonal temperature changes. The duration of gestation did not vary significantly from one group to the next. The number of corpora lutea and the number of pups surviving until the end of lactation showed some variations, but these also could not be related to dietary changes.

Measurements made for rats reared at room temperature were pooled and are presented in Table 4 together with the results for those reared at 34°C. Comparison of the two sets of data shows that, for all parameters, rats exposed to heat differed from those reared at more moderate temperatures. Moreover, unlike the rats reared at room temperature, dietary supplements affected the degree to which heat impaired growth and survival rate among pups during the first 21 days of extra-uterine life. Pentavite supplements produced no improvement in the weights achieved at 20 weeks

TABLE 5

RESULTS FOR RATS REARED AT 21, 27, AND 34°C AND FED PURIFIED DIETS AND PURIFIED DIETS SUPPLEMENTED WITH NATURAL PRODUCTS

Temp. (°C)	Diet	Initial No. of Rats	Weights at 20 Weeks (g) (±S.E.)	No. of Rats Mated	Duration of Gestation (days) (±S.E.)	No. of Corpora Lutea (±S.E.)	No. of Litters Surviving to 21 Days of Age	Litter Size at 21 Days Lactation (±S.E.)
21	Wheat–liver–casein	16	152±1·12	8	22·9±0·21	8·50±1·50	6	4·38±1·06
27	Casein	26	157±0·88	25	22·4±0·16	8·68±0·85	10	2·28†
	Liver–casein	10	188±1·41	9	22·8±0·17	9·33±1·41	7	4·33±1·00
	Wheat–liver–casein	16	164±1·12	11	22·9±0·06	8·55±1·28	9	4·55±0·90
34	Casein (group D)	26	128±0·88	25	22·4±0·10	7·40±0·85	4*	0·5†
	Liver–casein (group E)	6	145±1·83	6	22·5±0·20	7·83±1·73	1*	0·3†
	Wheat–liver–casein (group F)	16	146±1·12	10	22·7±0·09	7·80±1·37	3*	1·1†

* Significance of differences for survival of litters to 21 days of age for three groups of rats fed diets indicated are: groups D and E, $\chi^2 = 0{\cdot}334$, $0{\cdot}7 > P > 0{\cdot}5$; groups D and F, $\chi^2 = 0$, $P = 1{\cdot}0$; groups E and F, $\chi^2 = 0{\cdot}219$, $0{\cdot}7 > P > 0{\cdot}5$.

† The values for these groups showed a skew distribution due to the fact that a number of mothers lost entire litters. Because of this standard errors had no meaning and have not been quoted.

of age, but Vetemul and vitamin E supplements resulted in an increase of approximately 13%. On unsupplemented diets, no mother reared at 34°C was able to rear pups to 21 days of age, but when Pentavite supplements were added a small proportion of females was able to do so. Addition of Vetemul, vitamin E, and choline caused no significant improvement. On unsupplemented diets gestation was often prolonged, in one extreme case to as much as 28 days. Pentavite supplements returned the gestation period to near normal duration, but Vetemul, vitamin E, and choline had no further effect. Modification of the diet did not appear to affect the number of corpora lutea.

(b) Second Series of Experiments

Results for rats reared at 27 and 34°C and fed the three basic diets are presented in Table 5. The results for the rats reared at 21°C on the wheat–liver–casein diet have also been included in this table. Comparison of the results for rats reared at 27 and 34°C and fed similar diets shows that, in all cases, those reared at 34°C were lighter than those reared at 27°C. Moreover, the number of corpora lutea and the number of pups surviving until 21 days of age were significantly reduced in the group reared at 34°C.

At both temperatures, liver supplements improved growth rate and, at 27°C, this supplement increased the number of mothers capable of rearing pups to 21 days of age ($\chi^2 = 2{\cdot}42$, $0{\cdot}2 > P > 0{\cdot}1$). At 34°C liver supplements also increased the ability of the mothers to rear their young, although the improvement did not reach significant levels (Table 5). Liver supplements had no effect on the duration of gestation or on the number of corpora lutea in either group. Results for both the group reared at 27°C and that reared at 34°C indicate that the diet containing wheat as well as liver was in no way superior to that containing liver alone (Table 5).

When results for rats reared at 21°C and fed the wheat–liver–casein diet are compared with those given the same diet at 27 and 34°C, it would appear that although growth rates at 21°C were slower than those at 27°C, the duration of gestation, the number of corpora lutea, and the size of the litter at 21 days lactation were indistinguishable from those at 27°C. Examination of the data for weights at 20 weeks suggests that a temperature of close to 27°C is optimal for rat growth.

(c) Comparison of the Results from the First and Second Series of Experiments

When the results for the rats fed purified diets at 27°C (Table 5) are compared with those for rats fed stock diets at room temperature (Table 4), it is apparent that although the rats at 27°C grew more rapidly, those at room temperature produced more pups which survived until 21 days post-partum. It is not clear whether these differences were due to differences in diet or to differences in the diurnal temperature range to which the two groups were subjected.

Comparison of the results for rats fed stock diets at 34°C (Table 4) with those for rats given purified diets at the same temperature (Table 5) shows that growth was increased significantly by the addition of liver to the diet. The duration of gestation was shorter in all groups on purified diets than in those given stock diets, but there was no significant difference between the number of corpora lutea in the two groups,

nor was there a significant difference between the numbers of mothers able to rear young to 21 days of age ($\chi^2 = 0$). (This comparison was made between the pooled results for animals given the mash–Pentavite–Vetemul–vitamin E–choline diet and the cubes–mash–Pentavite–Vetemul–vitamin E diet, and the pooled results for animals given the liver–casein and wheat–liver–casein diet.)

V. Discussion

The most striking aspect of these experiments was that although the addition of vitamin supplements to stock diets and wheat and liver supplements to purified diets improved performance at 34°C, neither the growth rate nor the survival of young returned to control levels with any of the diets tried. Moreover, although differences were observed between the rats at 34°C on supplemented stock diets and those at the same temperature on purified diets, the similarities between the results for these two groups were greater than between those for rats at room temperature and at 34°C on stock diets, or between rats at 27 and 34°C on purified diets. This suggests that only a small part of the differences observed between rats reared at 34°C and those at lower temperatures are of nutritional origin.

The first series of experiments bears out earlier observations that animals acclimatized to high temperatures require higher concentrations of certain vitamins in the diet than animals at more moderate temperatures. The growth rate and reproductive performances of rats reared at room temperatures on unsupplemented stock diets were equal to those of rats fed all supplements, but this was not the case for rats reared at 34°C.

Though these experiments were not designed to investigate changes in requirements of specific vitamins, it is possible to make some assessment of the part played by each vitamin of the supplement in improving performance. Pentavite supplements did not increase weight gains but the Vetemul–vitamin E supplements did so. The additional vitamins supplied by this mixture were vitamins A, D, and α-tocopherol. All these are known to influence growth, so one or all of them could be responsible for the observed improvement. Although a Pentavite supplement failed to influence growth rate, it improved the number of pups surviving to 3 weeks of age at 34°C. This mixture is stated to supply vitamins A, D, thiamine, riboflavin, nicotinic acid, pyridoxine, sodium pantothenate, and ascorbic acid. The fact that the more massive doses of vitamins A and D supplied by the Vetemul–vitamin E mixture did not cause any further improvement in performance makes it unlikely that these were the active principles involved. Of the remaining vitamins in the mixture, only thiamine, riboflavin, and ascorbic acid supplies were increased significantly by addition of Pentavite to the diet. (The increases in pyridoxine, pantothenic acid, and nicotinic acid were so small when compared to the minimum requirements quoted by Brown and Sturtevant (1949) that they can be disregarded.) Of these, ascorbic acid is not thought to be an essential vitamin for the rat and there is no good evidence to suggest that requirements of this vitamin are increased at high environmental temperatures, especially if the body temperature is not elevated. Riboflavin is known to be essential for the rat but indications are that heat does not increase requirements for growth (Mills 1941, 1943*a*). This does not preclude the

possibility that increased amounts are required during reproduction. Thiamine requirements are known to be increased at high environmental temperatures (Mills 1941, 1943*b*). It seems likely therefore that this vitamin is the principal one involved in improving survivals at 34°C.

In the second series of experiments, the first diet tried was a purified one. Diets of this kind are known to be deficient in some element necessary for reproduction in the rat (Weisner and Yudkin 1958). Liver supplements improved growth and increased the number of mothers able to rear pups to 21 days of age at both 27 and 34°C. Wheat supplements caused no further improvement. Liver supplements increase supplies of several vitamins in addition to Weisner and Yudkin's liver factor. More experiments would be necessary to identify the effective factor in this supplement.

Of the diets tried in these experiments, Barastoc dog cubes supplemented with Pentavite and a Vetemul–vitamin E mixture and the rat mash made up with liver and casein appear to be the most likely diets to produce satisfactory growth and reproduction of rats kept at 34°C.

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