actively turning lobster *Palinurus vulgaris* and in the crab *Carcinus maenas* is identical, whether the animal is blinded or not. It has been concluded that this nystagmus is not a photokinetic response but a spontaneous action of central origin with possibly a steering component originating from the perception of movements of the limbs relative to the body[9].

It is hoped that further studies will reveal whether the function of the slow phase of the eye nystagmus described in the herring is restricted to the keeping of images on the ventral foveal region or whether it has an additional and more general function in photo orientation, and whether it is released exclusively by image movements or whether it has an active central component. In this way some contribution might be made towards a further understanding of the various aspects of 'optokinetic' movements in general. The described eye nystagmus of the herring has the advantage that the labyrinth and other receptors which might registrate rotations are precluded as sources of extra-retinal information since rotations are not involved in the genesis of this nystagmus. The delicacy of the animals, however, makes surgical interference rather precarious.

F. J. VERHEIJEN

Laboratory of Comparative Physiology, University of Utrecht, Holland, July 20, 1959.

Zusammenfassung

An Heringen, die in Aquarien schwimmen, kann ein Nystagmus beobachtet werden. Hierbei rotiert das Auge um eine Achse, die ungefähr senkrecht zur Pupillenebene steht. Der Nystagmus steht wahrscheinlich in Beziehung zu einer fovealen Struktur auf der ventralen Hälfte der Retina. Die Auslösung und Bedeutung «optokinetischer» oder «optomotorischer» Reaktionen wird diskutiert.

[9] S. DIJKGRAAF, Pubbl. Staz. zool. Napoli *28*, 341 (1956); Z. vgl Physiol. *38*, 491 (1956).

High-fat Diet and the Development of Obesity in Albino Rats

The relationship between the ratio of the main nutrients in the diet and the development of obesity in man is still the subject of extensive research[1]. Among experimental work concerned with this problem, the findings of MICKELSEN *et al.*[2] and of BARBORIAK *et al.*[3] who described the development of obesity in rats fed for a prolonged time on a high-fat diet, deserve special attention. However, it must be recalled that the hight-fat diet used in these authors' experiments had a higher energy value than the high-carbohydrate control diet and a lower percentage of energy derived from protein. It was therefore of interest to elucidate whether a high ratio of dietary fat *per se* leads to the development of obesity.

Young adult male rats (Wistar strain) were fed *ad libitum* for 44 weeks diets with different proportions of fat, carbohydrate, and protein. The diets contained the same ratio of a basal mixture; the nutrient administered in excess was provided in the form of an isocaloric amount of margarine, starch, or casein. The diets were supplemented by a 2·5% solution of agar to make them not only isocaloric (1 g = 2·3 cal), but also roughly isovoluminous. Except for the nutrient given in excess, the ratio of energy derived from the remaining two nutrients was practically equal (Table). These diets are a slight modification of the diets described in a previous work[4]. The rats were weighed once a week and the food intake of the different groups was measured. After 44 weeks on the experimental diets the animals were killed and the fat content of the whole eviscerated carcass was estimated by the method used by COHN *et al.*[5].

The results of the experiment are summarized in the table. We can see that the animals fed the high-fat and high-carbohydrate diet do not differ significantly in the weight increments nor in the amount of body fat. The growth of both groups was harmonious and the animals did not develop any signs of deficiency. In agreement with data in the literature[6], the weight increment and body fat were reduced in the group that were fed an excess of casein. The food intake per animal in all three experimental groups was practically equal. The caloric efficiency of the high-fat and the high-carbohydrate diet was thus the same while that of the high-protein diet was considerably lower. It must be mentioned that identical results

[1] J. MAŠEK, L. KŘIKAVA, and K. OŠANCOVÁ, Int. J. prophyl. Med. *2*, 132 (1958); Second Intern. Congress of Dietetics, Rome 1956, Coll. Communications, p. 271 (1958); Čs. gastroenterol. a výž. *13*, 246 (1959). – K. OŠANCOVÁ, Čs hygien.a *3*, 131 (1958).

[2] O. MICKELSEN, S. TAKAHASHI, and C. CRAIG, J. Nutr. *57*, 541 (1955).

[3] J. J. BARBORIAK, W. A. KREHL, G. R. COWGILL, and A. D. WHEDON, J. Nutr. *64*, 241 (1958).

[4] R. PETRÁSEK and P. FÁBRY, Arch. int. Physiol. Biochim. *66*, 610 (1958).

[5] C. COHN, D. JOSEPH, and E. SHRAGO, Metabolism *6*, 381 (1957).

[6] P. F. FENTON and C. J. CARR, J. Nutr. *45*, 225 (1951). – P. F. FENTON and M. T. DOWLING, J. Nutr. *49*, 319 (1953). – E. FALTOVÁ and O. POUPA, čs. gastroenterol. a výž. *10*, 229 (1956).

[7] P. FÁBRY, P. HAHN, O. KOLDOVSKÝ and J. MAŠEK (in preparation).

Table

Influence of Different Diets on Weight Gains and Body-Fat of Albino Rats after 44 Weeks of the Experiment

Group	Composition of diet			No. of animals	Initial weight (g)	Final weight (g)*	Weight gain (g)*	Fat content of eviscerated carcass (%)*
	Cal.% protein	Cal.% fat	Cal.% carbohydrate					
High-fat	14·9	70·0	15.1	14	191 ± 6·5	447 ± 18·2	256 ± 16·0	20·45 ± 1·23
High-carbohydrate	15·2	10·5	74·3	13	189 ± 4·4	421 ± 18·3	232 ± 13·6	19·88 ± 1·58
High-protein	73·1	11·5	15·4	14	191 ± 6·9	314 ± 12·1	123 ± 10·1	10·69 ± 0·99

The values are given in the averages of the groups (weights as the nearest whole number) ± S.E.

* The values of the high-fat and high-carbohydrate group do not differ significantly. The difference between the high-protein group and the remaining two groups is statistically significant for $P < 0{\cdot}01$.

were obtained in a preliminary experiment with groups of 7, 6, and 8 rats, having initial weights of ca. 90 g and fed these contrasting diets for 20 weeks.

The results of our experiments indicate clearly that the high ratio of dietary fat *per se* did not lead to the development of obesity in young adult rats. In subsequent experiments we revealed that a high-fat diet does not produce obesity in Wistar rats even when administered from the 18th or 30th day after birth, respectively[7]. In the experiments of MICKELSEN *et al.*[2] and BARBORIAK *et al.*[3], other factors in addition to the percentage of calories provided by fat must have been at play which led to an absolute or relative hyperphagia and finally to obesity of the experimental animals. For instance, the high-fat diet used in their experiments contained more calories (per weight or volume) then the control diet and the energy value derived from protein was substantially smaller. It is also possible that the strains used by these workers (Osborne-Mendel and Sprague-Dawley resp.) respond to the high-fat diet in a different manner.

J. MAŠEK and P. FÁBRY

Institute of Human Nutrition, Prague (Czechoslovakia), July 15, 1959.

Zusammenfassung

Männliche, geschlechtsreife Wistar-Ratten wurden 44 Wochen lang mit isokalorischen Diäten gefüttert, die entweder einen erhöhten Fett- oder Kohlehydrat- bzw. Eiweissanteil enthielten. Zwischen den eine fettreiche und eine kohlehydratreiche Diät erhaltenden Tieren zeigten sich keine statistisch bedeutsamen Unterschiede in bezug auf ihre Gewichtszunahme und den Gesamtanteil des Körperfettes. Diejenigen Tiere, die eine Diät mit erhöhtem Kaseingehalt erhielten, nahmen in Übereinstimmung mit den Literaturangaben weniger an Gewicht zu und lagerten auch weniger Körperfett ab. Wie aus unseren Ergebnissen hervorgeht, muss ein hoher Fettanteil in der Diät von sich aus noch nicht zur Fettsucht bei Ratten führen.

The Specific Gravity of Liver and its Relation to the Fat Content following High Fat Diets and Carbon Tetrachloride Poisoning

Fat is a normal constituent of all livers, and its concentration is increased in many diseases and metabolic changes, e.g. in toxic hepatitis, cirrhosis, spontaneous diabetes mellitus, and acetonemia. Parasitic infections can cause an increase in the amount of fat but this may be more localised than in toxic hepatitis. During an investigation by a colleague (Dr. J. S. WILKINSON) on spontaneous diabetes in dogs, consideration was given to a routine and rapid estimation of fat in biopsy and small *post-mortem* samples. Several workers have investigated chemical methods notably BILLING, CONLON, HEIN, and SCHIFF[1] who have described a microtechnique and its application to the investigation of human liver disease. Although this method is elegant, it requires rather specialised and skilled techniques, so it was decided to investigate the possibility of establishing a relation between the specific gravity of a liver sample and its fat content. Preliminary experiments indicated that *fresh* liver samples could be titrated with benzene and chloroform[2,3] to give fairly precise measurements of their specific gravities with little loss of lipoid material into the solvents during the process. This led to the planning of two major experiments. In the first a group of thirty mice were fed *ad libitum* quantities of a butter fat diet containing 3% of a salt mixture[4] for 4–5 days. Each day five animals were sacrificed by light etherisation and exsanguination, and the livers removed. These were weighed rapidly in air, in benzene and then titrated with chloroform. Three small portions (5–20 mg) were removed at random and also titrated. The portions of liver were combined, dried (100–110°C, 18 h) weighed, and extracted several times with a 50·50 mixture of diethyl ether: petroleum ether (B. P. 40–60°C) to determine the fat content. It was found that significant regressions could be extracted from the *probit* per cent fat per dry weight of the liver and the specific gravities determined by the three methods. Further, it was found that there was no significant difference between the three regressions (Table I). Regressions on group values gave similar results (Table II).

The gradient diffusion method for specific gravity measurement was also investigated. This gave similar results. In preparing a gradient of suitable range mixtures of bromobenzene and charcoal decolourised domestic

[1] B. H. BILLING, H. J. CONLON, D. E. HEIN, and L. SCHIFF, J. clin. Invest. *32*, 214 (1953).
[2] D. G. HARVEY, Brit. Vet. J. *113*, 52 (1957).
[3] D. M. G. ARMSTRONG and A. E. HAWKINS, Physics Biol. Med. *2*, 338 (1958).
[4] R. B. HUBBELL, L. B. MENDEL, and A. J. WAKEMAN, J. Nutr. *14*, 273 (1937).

Table I

Regressions (*b*) of probit per cent liver fat (dry weight) and specific gravity. Feeding experiments

Method	*n*	*b* ± S. D.	*P*	Equation
1. Weighing Air/Benzene	25	– 0·0180 ± 0·0014	< 0·001	$y = 4·76 - 0·018\,x$
2. Titration: Whole liver	25	– 0·0144 ± 0·0090	< 0·001	$y = 4·59 - 0·0144\,x$
3. Titration: Mean three pieces .	25	– 0·0162 ± 0·0011	< 0·001	$y = 4·65 - 0·0162\,x$
4. Diffusion Gradient. Separate Experiment	29	– 0·0200 ± 0·0001	< 0·001	$y = 5·13 - 0·020\,x$

Notes.—Regressions calculated on *last two* figures of Specific Gravity determinations e.g. 1·072, and *first three* of the probit values, e.g. *4·3214*.

Comparison of the residual variances of 1, 2, 3, namely 0·0342, 0·0249, and 0·0268 reveals no significant difference at the 5% level, therefore an overall regression has been calculated. This is – 0·0159 ($P < 0·001$) and gives an equation:

$$y = 4·60 - 0·0159\,x.$$

Comparison between 1 and 4 reveals no significant difference.