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.

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

Notes. – Regressions calculated on *last two* figures of Specific Gravity determinations e.g. 1·072, and *first three* of the probit values, e.g. *4·321*4.

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 $-0{\cdot}0159$ ($P < 0{\cdot}001$) and gives an equation:

$$y = 4{\cdot}60 - 0{\cdot}0159\,x.$$

Comparison between 1 and 4 reveals no significant difference.

Table II

Regressions (b) of probit per cent liver fat (dry and wet weight) and specific gravity, with ($n = 5$) and without ($n = 4$) 96 h value

Type of Measurement	n	$b \pm$ S. D.	P
Dry Weight *A*	5	-0.0180 ± 0.0022	$< 0.01 > 0.001$
A	4	-0.0171 ± 0.0023	$< 0.05 > 0.01$
Wet Weight *A*	5	-0.0220 ± 0.0040	$< 0.05 > 0.01$
A	4	-0.0200 ± 0.0040	$< 0.05 > 0.01$
Dry Weight *B*	5	-0.0176 ± 0.0012	< 0.001
B	4	-0.0172 ± 0.0013	$< 0.01 > 0.001$
Wet Weight *B*	5	-0.0220 ± 0.0040	$< 0.05 > 0.01$
B	4	-0.0200 ± 0.0040	$< 0.05 > 0.01$
Dry Weight *C*	5	-0.0155 ± 0.0022	$< 0.01 > 0.001$
C	4	-0.0146 ± 0.0013	$< 0.01 > 0.001$
Wet Weight *C*	5	-0.0190 ± 0.0036	$< 0.05 > 0.01$
C	4	-0.0170 ± 0.0001	< 0.001

Notes.—Regressions calculated as in Table I.

A = Specific Gravity by weighing, *B* = by Titration of whole liver, *C* = by Titration of Pieces.

Table III

Regressions (b) of probit per cent liver fat (dry weight) and specific gravity; log dose CCl_4 specific gravity and probit per cent liver fat

Type of Regression	n	$b \pm$ S. D.	P	Equation
1. Six groups of rats dosed with CCl_4 and two control groups.				
Specific Gravity (x), Probit liver fat, (y); x and y are mean values of groups	8	-0.0284 ± 0.0038	< 0.001	$y = 6.86 - 0.028\,x$
2. Four groups of rats dosed 0·1, 0·2, 0·4, and 0·8 ml CCl_4/kg; five doses.				
Log (dose × 10) (x), Specific Gravity (y)	15	-19.84 ± 5.00	< 0.01 > 0.001	$y = 101.64 - 19.8\,x$
Log (dose × 10) (x), Probit per cent fat (y)	15	$+0.50 \pm 0.16$	< 0.01 > 0.001	$y = 3.11 + 0.50\,x$

paraffin were used. These had greater specific gravities than those normally recommended for the estimation of plasma proteins[5].

In the second experiment the effects of a toxic agent (CCl_4) on the liver fat of rats were studied. Two groups of four rats per group were given a single dose of 1·0 ml/kg CCl_4 by intraperitoneal injection. Four groups were given 0·1, 0·2, 0·4, and 0·8 ml/kg on alternate days over a period of ten days. The animals were sacrificed, the liver specific gravities and fat determined as before. Good regressions were obtained for specific gravity and liver fat, log dose CCl_4, liver fat and specific gravity (Table III).

The experiments described suggest that there are considerable possibilities in the development of this technique for the assessment of liver fat in disease. How accurately a single determination can be made to forecast the fat content of the whole liver remains to be further investigated, but the degree of prediction is probably similar to that suggested by BILLING *et al.*[1]. Further studies are in progress to improve the accuracy of the technique and to investigate its applicability in the investigation of disease in animals.

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Résumé

Il existe un rapport étroit entre le poids spécifique et la teneur en matières grasses du foie. On peut le démontrer en utilisant des animaux empoisonnés avec du tétrachlorure de carbone.

[5] E. A. KABAT and M. M. MEYER, *Experimental Immunochemistry* 1st Ed. (Charles Thomas, Springfield (Ill.) 1948).