DIET AND TUBERCULOSIS.

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The following series of experiments was carried out during the past five or six years and though the results have been largely of an inconclusive character, they should, we think, be put on record.

The experiments of Zilva (1919) having shown that the production of certain immune bodies is not influenced by the absence or presence from the animal's diet of the fat soluble vitamins, it was decided to investigate the effect of controlled diets on the course of an animal infection, tuberculosis being chosen in order to work with disease in a chronic form. Guinea-pigs were used in the first place, but later rats were employed because of the facility with which abnormal diets can be fed to them.

When this work was first undertaken, the bearing of the accessory food-factor content of diet upon disease had been little investigated. Since then Gloyne and Page (1921) have shown that it is not possible to produce macroscopic tubercular lesions in rats even when fat-soluble vitamins are withdrawn from their food. Werkman (1923) has found that rats and rabbits are rendered more susceptible to anthrax and pneumococcus infections by diets lacking fat-soluble vitamins. Mouriquand and his fellow-workers (1923) declared that the tubercular lesions occurring in guinea-pigs placed on a diet deficient in fat-soluble vitamins were not greater than those in animals normally fed. By feeding guinea-pigs with cod-liver oil, Smith (1923) was not able to prolong the life of guinea-pigs inoculated with tubercle bacilli neither was he able to improve their weight curves or curtail the extent of the disease. Cramer and Kingsbury (1924) injected two vitamin-deficient and two normal rats intraperitoneally with tubercle bacilli; 4 weeks later the two deficient rats showed enlarged mesenteric glands containing tubercle bacilli, whereas the two controls did not.

Abundant and restricted diets.

In a preliminary experiment, a complete normal diet given in abundance was contrasted with a similar one given in restricted quantities; the animal chosen was the guinea-pig and the diet consisted of oats and bran (one to two by volume), cabbage and water. In the case of the animals on the restricted diet the amount of food was quantitatively regulated by the daily weight of the animal, just sufficient being given for each one to keep its original weight level. Vitamin C was in all cases adequate. In this way animals of the same age but of different weight were contrasted in the two groups.

After $2\frac{1}{2}$ months of these diets the guinea-pigs in the abundant-diet group weighed on the average 500 gm., while those in the restricted-diet group remained at their original weight, viz. between 250 and 300 gm. All the guinea-pigs were then inoculated subcutaneously in the thigh with 0·1 mg. of living tubercle culture, half of each dietetic group receiving a virulent human strain "May," and half being given an attenuated bovine strain "H 149," both of which cultures were kindly supplied by Dr A. S. Griffiths. The following is a record of the deaths in the four different groups.

Table I.

Guinea-pigs infected with 0·1 mg. of	Diet	Days after infection on which animals die	Average length of life
Virulent human T.B.	Abundant	52, 55, 58, 68, 72, 73, 76, 82, 95, 107, 113, 200	88 days
	Restricted	31, 37, 38, 38, 49, 49, 51, 53, 55, 73, 80	50 ,,
Attenuated bovine T.B.	Abundant	74, 92, 103, 132, 135, 201, 228, 256, 256, 256, 256, 304, 327	202 ,,
	Restricted	38, 53, 60, 60, 61, 62, 65, 84, 84, 135, 142, 202	87 ,,

The length of life in the restricted-diet animals is seen to be roughly half that of the abundant-diet animals and though the death of a tuberculous guinea-pig is sometimes finally due to a superimposed Salmonella or other infection the death-rate gives evidence of the deleterious effect of a restricted diet on the course of tubercular disease in the guinea-pig. In 40 of the 48 animals, blood was plated from the heart after death and a septicaemia demonstrated in 6 cases, twice due to *B. aertrycke*, thrice to *B. gaertner* and once to both combined.

Chaulmoogra and cod-liver oil in vitro.

Cod-liver oil has long been an important item in the treatment of tubercular processes, while chaulmoogra oil, considered effective in leprosy, has, by analogy, been suggested for tuberculosis. In the form of its sodium salt, chaulmoogric acid has been proved (Walker and Sweeney, 1920; Lindenburg and Pestana, 1921) to inhibit, even in very high dilutions, the *in vitro* growth of tubercle bacilli, while non-acid-fast bacteria are capable of multiplying in quite strong concentrations; this effect of chaulmoograte is shown in Table II, where it is also seen that the sodium salts of other fatty acids are almost equally capable of the same inhibitory action.

The method of experimentation was as follows. To prepare the soaps 50 gm. oil were added to 40 c.c. NaOH (560 gm. per litre) and 40 c.c. absolute alcohol and heated on a water-bath till the mixture assumed the consistence of a paste: by then most of the alcohol was evaporated. The paste was then dissolved in 1500 c.c. of distilled water and boiled for 60–90 minutes at constant volume to remove the last traces of alcohol. The fatty acids were precipitated with 4N H_2SO_4 and washed with boiling distilled water until the washings reacted neutral to litmus.

The soaps were finally prepared by melting a known quantity of the fatty acids, dissolving it in excess of absolute alcohol while hot, and adding sodium

hydroxide cautiously until the solution was just alkaline to litmus and acid to phenolphthalein. The alcohol was then evaporated on the water bath and the residue, when dry, was dissolved in the requisite quantity of distilled water to make a 10 per cent. soap solution. As the oils, after saponification, were not extracted with ether, the morrhuates were rich in the fat-soluble vitamins.

A tubercle strain which grows easily in the depths of a glycerine broth medium having been selected and a glycerine broth which favours such growth (for some undiscovered reason, not every glycerine broth is favourable) having been chosen, a series of dilutions of the various sodium salts was made in 5 c.c. quantities of the glycerine broth and a specified number of capillary drops of a homogeneous emulsion of the tubercle bacilli was inoculated into each tube which was then sealed and placed at 37°. After 2–3 weeks the tubes may be read off, the lower dilutions showing inhibition of growth, the higher ones a flocculent deposit of tubercle bacilli.

Table II.

Sodium soap from	Dilution at which growth is still inhibited
Chaulmoogra oil	1/500,000–1/1,000,000
Cod liver oil	1/200,000–1/500,000
Peanut oil	1/200,000–1/500,000
Linseed oil	1/100,000–1/200,000
Cottonseed oil	1/100,000–1/200,000
Coconut oil	1/100,000–1/200,000
Beef suet	1/100,000
Palm oil	1/100,000
Cocoa oil	1/100,000

Chaulmoogra and Cod-liver oil in vivo.

To test the effect of chaulmoogra oil and the fat-soluble vitamins of cod-liver oil on the course of infection with tubercle bacilli, rats were chosen, being animals suitable to dietetic experimentation. Infection was initiated by the intraperitoneal inoculation of 10 mg. of culture of an avian tubercle bacillus "C," which was seen to cause, in a certain number of cases, a well-defined omental tumour consisting of bacilli and proliferating tissue and reaching sometimes the size of the spleen.

It was hoped that this macroscopic evidence of infection would serve as an index of infection.

The rats chosen for these experiments were fed on the following basal diet, which was the one used throughout all the experiments recorded here. It caused cessation of growth in about three weeks but permitted of survival for the period of experimentation, most rats showing xerophthalmia in varying degree.

Heated caseinogen.
Starch.
Hardened cotton seed or sesame oil.
Marmite.
Salt mixture[1].
Decitrated lemon juice.
Water.

[1] No. 185, McCollum, Simmonds and Pitz (1917).

https://doi.org/10.1017/S0022172400009062 Published online by Cambridge University Press

In the earlier experiments, no fat-soluble vitamins at all were given. In the later ones, a bare supply of cod-liver oil, sufficient to give a normal growth curve was added, so that the effect of chaulmoograte both without and in the presence of an adequate supply of fat-soluble vitamins might be tested and compared with that produced by a diet superabundant in these accessory food substances. The oil used for the last experiments of these series was shown by Steenbock's method (1924) and by the calcification test to contain both vitamin A and vitamin D in large amounts.

For those rats that were to receive fat-soluble vitamins in superabundance, $\frac{1}{4}$ gm. of cod-liver oil per day was added, while those to receive sodium chaulmoograte, or in some cases, sodium morrhuate or sodium stearate (as a control), were given 1 c.c. of the soap in 10 per cent. strength. Care was taken that these substances were completely consumed. The various combinations of diets with and without fat-soluble vitamins and with and without sodium chaulmoograte or sodium stearate were fed to rats for a month previous to inoculation: 4 weeks after the intraperitoneal inoculation of 10 mg. of T.B. the animals were killed and examined for macroscopic signs of tubercle infection which, except for an occasional nodule at the site of inoculation, were confined to the omentum. According to the size of the omental infection the animal was recorded as having a degree of infection equal to 2, 1, $\frac{1}{2}$ or 0; the average degree of infection in a group of animals could be thus roughly ascertained.

Table III.

No. of rats	Diet	In addition	Degree of infection as measured by the average amount of omental tumour
Exp. 6			
10	− F.S.V.*	—	1·45
12	− F.S.V.	Chaulmoograte	1·5
10	− F.S.V.	Stearate	1·5
12	− F.S.V.	Morrhuate (fat soluble vitamins not extracted)	0·87
Exp. 7			
4	+ F.S.V.	—	1·75
4	+ F.S.V.	Chaulmoograte	1·75
2	+ F.S.V.	Stearate	1·0
4	+ F.S.V.	Excess of cod-liver oil	0·87
4	+ F.S.V.	Ditto + chaulmoograte	0·62
Exp. 7 *a*			
6	+ F.S.V.	—	0·5 (!)
4	+ F.S.V.	Chaulmoograte	2·0
8	+ F.S.V.	Stearate	1·12
2	+ F.S.V.	Excess of cod-liver oil	0·25
Exp. 9			
19	+ F.S.V.	—	1·18
17	+ F.S.V.	Chaulmoograte	1·79

* The fat-soluble vitamins recorded in this column are those contained in the minimal amount of cod-liver oil needed for producing a prolonged normal growth curve, *i.e.* generally about 1 drop of the oil as used in these experiments.

Summary of experiments with chaulmoogra oil.

While the irregularities, inseparable from biological observations of this nature, are great, Table III may be held to demonstrate that no favourable influence is exerted by sodium chaulmoograte on the course of tuberculous infection in the rat, but that a surplus of fat-soluble vitamins does inhibit the development of this omental infection to some extent. When Exps. 4–7 *a* were considered together and all animals receiving a bare supply of cod-liver oil compared with those receiving an abundance, the facts appeared to justify this conclusion.

Table IV.

Exps. 4, 5, 6, 7, 7*a*	No. of rats	Diet	Degree of infection as measured by the average amount of omental tumour
	91	Meagre supply of cod-liver oil (just sufficient for *growth purposes*)	1·29
	25	Abundant supply of cod-liver oil	0·98

To test this point more explicitly, Exp. 8 was devised in which one group of rats received in addition to their basal diet, the minimal amount of cod-liver oil necessary for prolonged normal growth, viz. 1 drop per day, and the other group was given a more than ample supply, viz. $\frac{1}{2}$ gm. (about 25 drops) for the first ten days and subsequently because of the threatened interference with digestion, $\frac{1}{4}$ gm. The result, as Table V shows, was encouraging.

Table V.

Exp. 8	No. of rats	Diet	Average amount of omental tumour
	15	Cod-liver oil just adequate for growth (1 drop)	1·75
	20	Cod-liver oil in excess ($\frac{1}{4}$ gram)	0·92

An exact repetition in Exp. 9 failed, however, to give confirmation as Table VI shows.

Table VI.

Exp. 9	No. of rats	Diet	Average amount of omental tumour
	19	Cod-liver oil just adequate for growth (1 drop)	1·18
	18	Cod-liver oil in excess ($\frac{1}{4}$ gram)	1·19

As Exp. 9 had been carried out with a cod-liver oil different from that used in Exp. 8 and possibly in some way at fault, the experiment was repeated once again with still another batch of oil, there being no more of that fed in Exp. 8.

Table VII.

Exp. 10	No. of rats	Diet	Average amount of omental tumour
	12	1 drop of cod-liver oil daily	0·4
	11	$\frac{1}{4}$ gram of cod-liver oil daily	0·27

A difference in the two groups is here again noticeable, although the extent to which the omentum was affected was in both groups less than usual.

Three more experiments on the same lines were carried out (certain details being varied, such as amount of inoculum, which was increased to 20 mg., and time of inoculation, which was alternatively placed before and after feeding), but no real return to the previous heavier infections took place, as is seen in Table VIII.

Table VIII.

Exp. 11	No. of rats	Diet	Average amount of omental tumour
	13	No cod-liver oil	0·08 (!)
	19	Cod-liver oil just adequate for growth (1 drop)	0·14
	19	Cod-liver oil in excess ($\frac{1}{4}$ gram)	0·16
Exp. 12			
	18	Cod-liver oil just adequate for growth (1 drop)	0·8
	20	Cod-liver oil in excess ($\frac{1}{4}$ gram)	0·4
Exp. 13			
	15	Cod-liver oil just adequate for growth (1 drop)	0·1
	16	Cod-liver oil in excess ($\frac{1}{4}$ gram)	0·03

The avian strain "C" appeared to have lost to some extent its power of producing omental infections.

As it had on a previous occasion been found that human and bovine tubercle bacilli were equally capable of causing omental tumours in the rat, a further experiment, recorded in Table IX, was carried out, similar to the foregoing, except that this time the bovine strain "B 4" was used, but there was no return to the 1·5 averages of the earlier experiments.

Table IX.

Exp. 14	No. of rats	Diet	Average amount of omental tumour
	11	Cod-liver oil just adequate for growth (1 drop)	0·54
	15	Cod-liver oil in excess ($\frac{1}{4}$ gram)	0·66

Summary of experiments with cod-liver oil.

A survey of Tables III to IX indicates that a majority of the experiments, viz. 4–7 *a*, 8, 10, 12 and 13, favours the idea that a superabundance of cod-liver oil in the diet of the rat diminishes the extent of tubercle infection, as measured by the formation of omental tumours. However, a considerable minority, viz. Exps. 9, 11 and 14, yields the theory no support whatever. It is, therefore, very likely that the development of these omental infections depends to some extent at least on other factors not controlled in these experiments.

Tuberculin shock in rats.

It is well known that rats infected with tubercle bacilli are not sensitive to tuberculin and early in this series of experiments three rats living on a diet deprived of fat-soluble vitamins and infected with tubercle bacilli were found to be undamaged by tuberculin injections. Recently Smith (1926) has declared that lack of fat-soluble vitamins renders rats susceptible to tuberculin shock. As his results conflicted with those of our early experiment and, because of

the small size of this experiment and its having been performed with an avian bacillus, it was now repeated.

A number of rats was divided into three groups—9 belonging to Group A and 19 belonging to Group C received no fat-soluble vitamins except a very occasional drop of cod-liver oil, necessary for preserving life over the long period of experiment (that the deficiency was maintained is evidenced by the weight curves; members of Group C, for instance, had an average weight of 50 gm. at the beginning of the experiment and of 89 gm. at its termination after 11 weeks); 19 belonging to Group B were given a complete diet. After 3 weeks Groups B and C were inoculated intraperitoneally with 10 mg. of a human tubercle culture and 8 weeks after infection all the animals were given intraperitoneally an injection of Frankfort standard tuberculin equal to 0·35 c.c. of the undiluted fluid. This large dose produced immediate symptoms of distress in all groups regardless of whether they were infected with tubercle or not; from this, however, all animals recovered except 1 rat in Group A, which had been on a deficient diet but not been inoculated, and 1 rat in Group C which had been inoculated and on a deficient diet. Both these rats died within 24 hours but without the *post mortem* appearances indicative of tuberculin shock.

Summary of tuberculin shock experiments.

Sensitiveness to tuberculin has not developed in rats infected with tubercle bacilli and at the same time fed on a diet lacking fat-soluble vitamins.

Ultra-violet irradiations.

In two of the previous experiments groups of rats which had been exposed to ultra-violet rays were also included. The rats were irradiated daily for a quarter of an hour at a distance of 10 inches from a 4 amp. tungsten arc. The food of all four groups consisted of the basal diet already described + 1 drop of cod-liver oil per day. The results are given in Table X.

Table X.

Exp. 10	No. of rats	Ultra-violet Irradiation	Average amount of omental tumour
	12	–	0·42
	12	+	0·17
Exp. 12			
	18	–	0·8
	20	+	0·5

Summary.

Possibly, therefore, irradiation has an inhibitory influence on the course of tubercle infection in the rat.

Leucocyte counts in irradiated and cod-liver oil dieted rats.

Taylor and Murphy (1917) causing a leucopaenia in mice by the use of X-rays, at the same time rendered them more susceptible to tubercle infection; on the other hand, by inducing a leucocytosis they could confer on the animals an increased resistance which radiation could withdraw from them again. Mottram and Kingsbury (1924) used X-rays on rats with the same result.

In an attempt to explain on these lines what looked, in a certain number of experiments, like a beneficial effect of both cod-liver oil and ultra-violet irradiation, white corpuscle counts were made in Exps. 9, 10, 11 and 12. The tail-count method was employed and the blood was taken before the daily feeding or irradiation had taken place. To ensure adequate bleeding the rats were placed in a 37° C. incubator for 6–10 minutes, care, however, being taken that all the animals to be compared were exposed to the heating for the same length of time, for this influences the white count considerably as others (Murphy and Sturm, 1919) have already noted.

Table XI.

Exp.	No. of rats	Diet, etc.	Average leucocytic count of each Group at various times during the experiments	
9	18	1 drop cod-liver oil	One month on diet before inoculation ...	8,600
			Two weeks after inoculation	8,500
			Four weeks after inoculation	10,200
	18	¼ gm. cod-liver oil	One month on diet before inoculation ...	11,300
			Two weeks after inoculation	10,400
			Four weeks after inoculation	14,700
	17	1 drop cod-liver oil + chaulmoogra	One month on diet before inoculation ...	9,600
			Two weeks after inoculation	8,100
			Four weeks after inoculation	10,800
10	12	1 drop cod-liver oil	Two months on diet, 4 weeks after inoculation	9,200
	11	¼ gm. cod-liver oil	Two months on diet, 4 weeks after inoculation	11,600
	12	1 drop cod-liver oil + irradiation	Two months on diet, 4 weeks after inoculation	13,300
11	19	1 drop cod-liver oil	Before putting on diet	6,570
			One month on diet, before inoculation ...	7,650
	19	¼ gm. cod-liver oil	Before putting on diet	7,080
			One month on diet, before inoculation ...	9,060
	13	No cod-liver oil	Before putting on diet	6,120
			One month on diet, before inoculation ...	7,330
12	18	1 drop cod-liver oil	One day on diet	4,090
			One month on diet before inoculation ...	4,630
	18	¼ gm. cod-liver oil	Before putting on diet	3,885
			One month on diet before inoculation ...	6,560
	17	1 drop cod-liver oil + irradiation	One week on diet and rays	7,137
			One month on diet before inoculation ...	8,080

Summary of leucocytosis observations.

An influence on the leucocytic count of a greatly increased amount of cod-liver oil in the diet seems, therefore, to be established and the effect of a daily ultra-violet irradiation also, for in every case a definite, though slight, leucocytosis is observable. But seeing that an equally good leucocytosis was observed in Exps. 9 and 11, where cod-liver oil failed to protect, as in Exps. 10 and 12, where it apparently did, no correlation between leucocytosis and diminished amount of omental tubercle can be herewith established.

Other workers (Cramer, Drew and Mottram, 1921) have found that a total lack of fat-soluble vitamin in the diet did not appreciably alter the white-blood-cell count of rats, and in the one experiment (Exp. 11) where the effect of a *diet containing no vitamin* is compared by us with that of *one just adequate* in that respect, this point is confirmed, there being no noteworthy difference in the two counts.

General Summary.

Guinea-pigs living on a diet restricted in quantity but not deficient in vitamin C survived inoculation with T.B. but half as long as others which had received a similar diet in abundance.

Sodium chaulmoograte did not inhibit the development of the omental tumour that appears in rats after intraperitoneal inoculation with tubercle bacilli.

On the other hand, there was some evidence for assuming that a large excess of fat-soluble vitamins in the diet, as supplied by cod-liver oil, inhibits the formation in rats of these tuberculous tumours, but such evidence was by no means conclusive.

Similar inhibition of omental infection was obtained on exposing rats to ultra-violet light.

Ultra-violet irradiation or the inclusion of *large* amounts of cod-liver oil in the diet of the rats produced a slight but constant leucocytosis.

No evidence was obtained that lack of fat soluble vitamins in their diet renders tubercle infected rats susceptible to tuberculin shock.

Acknowledgment: One of us (S. S. Z.) is indebted to the Medical Research Council for a personal grant.

REFERENCES.

Cramer, W., Drew, A. H. and Mottram, J. C. (1921). *Lancet*, ii, 1202.
Cramer, W. and Kingsbury, A. N. (1924). *Brit. J. Exp. Path.* **5**, 300.
Gloyne, S. R. and Page, D. S. (1921). *Tubercle*, **3**, 1.
Lindenburg, A. and Pestana, B. (1921). *Zeitschr. f. Immunität.* **32**, 66.
McCollum, E. V., Simmonds, N. and Pitz, W. (1917). *J. Biol. Chem.* **29**, 521.
Mottram, J. C. and Kingsbury, A. N. (1924). *Brit. J. Exp. Path.* **5**, 220.
Mouriquand, C., Michel, P. and Bertoye, P. (1923). *C. R. Soc. Biol.* **88**, 1043.
Murphy, J. B. and Sturm, E. (1919). *J. Exp. Med.* **29**, 35.
Smith, M. I. (1923). *Am. Rev. Tub.* **7**, 33.
—— (1926). *U.S.A. Pub. Health Rep.* **41**, 767.
Steenbock, Nelson and Black (1924). *J. Biol. Chem.* **72**, 275.
Taylor, H. D. and Murphy, J. B. (1917). *J. Exp. Med.* **25**, 609.
Walker, E. and Sweeney, M. (1920). *J. Infect. Dis.* **26**, 238.
Werkman, C. H. (1923). *Ibid.* **32**, 255.
Zilva, S. S. (1919). *Biochem. J.* **13**, 172.

(*MS. received for publication* 21. II. 1927.—Ed.)