glutination. By Schiff's staining it was found that the receptor protein is a glycoprotein. The molecular weight of the receptor protein as found by the gel filtration technique is approximately 40,000 daltons.

In the seed germination experiment, as the seeds germinate the receptor protein is liberated into the surrounding medium; there is a specific period, i.e. the 3rd and 4th day, when a moderate amount of lectin is available in the medium and the *Rhizobia* are in the competence stage, and at this particular time the binding takes place. Receptor protein released after this period is of no use, once the binding is over. The maximum amount is released on the 5th and 6th day, after which the leaching of receptor protein declines. In the nodulation test the highest nodulation obtained is in seeds inoculated on the 3rd and 4th day. Thus the correlation between release of receptor protein from germinating seeds, FITC cell binding of *Rhizobium* and nodulation indicates that the best period for interaction lies between the 3rd and 4th day (fig. 2). Nodulation and cell binding by the FITC technique were shown only by homologous *Rhizobia* the heterologous strains failed to show it (table 3). These results are in agreement with those of Bohlool and Schmidt[4], Bhuvaneswari et al.[3] and others, indicating that lectin receptor protein may have the role of controlling specific binding in *Rhizobium* - legume root nodule symbiosis.

Table 3. Nodulation and cell binding ability of different *Rhizobium* cultures

Rhizobium strain	Nodulation per plant	% R. cell binding by FITC technique
1. M-13	+	65
2. D211	−	0
3. M-03	+	54
4. R. phaseolus	−	0
5. M-4015	+	60
6. R. trifolii	−	5
7. M-1	+	44
8. M-4013	+	52
9. M-11	+	50
10. M-5	+	45
11. M-7	+	48
12. M-17	+	41
13. R. cowpea	−	0
14. Sb-16	−	0

1 Lis, H., and Sharon, N., A. Rev. Biochem. *42* (1973) 541.
2 Bauer, W.D., in: Genetic engineering for nitrogen fixation, p. 283. Ed. A. Hollander. Plenum Press, New York 1977.
3 Bhuvaneswari, T.V., Pueppke, S.G., and Bauer, W.D., Pl. Physiol. *60* (1977) 486.
4 Bohlool, B.B., and Schmidt, E.L., Science *185* (1974) 269.
5 Dazzo, F.B., and Hubbell, D.H., Appl. Microbiol. *30* (1975) 1017.
6 Dazzo, F.B., Pl. Soil *43* (1975) 713.
7 Chen, A.T., and Phillips D.A., Physiologia Pl. *38* (1976) 83.
8 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
9 Shuster, L., Meth. Enzym. *22* (1971) 412.
10 Tobiska, J., Die phythamagglutinine Hämatologie und Blutransfusionswesen, vol. 3, p. 169. Adaemic Verlag, Berlin 1964.
11 Vincent, J.M., I.B.P. handbook No. 15. Blackwell, Oxford 1970.
12 Stacey, Gary, Paau, A.S., and Brill, W.J., Pl. Physiol. *65* (1980) 67.

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Monoamine oxidase A deficit in liver of germ-free rats

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Summary. Monoamine oxidase A but not B was found to be significantly decreased in the liver of germ-free compared with conventional rats. This change is the opposite of that found in germ-free chicks. Rat brain enzyme was not affected by the germ-free state.

Phillips et al.[3] reported significantly higher monoamine oxidase (EC 1.4.3.4; MAO) activity in the liver of the germ-free chicks compared with conventionally-bred controls. Böhm et al.[4] confirmed this difference for both the A and B forms of MAO in liver, but were unable to find any change in brain MAO activity between germ-free and conventional animals of this species. In the present study we have attempted to determine whether a similar pattern was present in the rat, where gross anatomical changes have been noted in the gut of the germ-free animal[5]. In the event, a striking difference in enzyme activity was observed between the 2 species.

Method. Male rats (268–383 g) of the Lister hooded strain were used. They were born and reared germ-free in Gustafsson stainless steel isolators[6] until weaning. Part of each litter was retained in the germ-free isolators and the rest of the animals were removed to a conventional environment where the conditions of lighting, humidity and temperature were matched to those obtaining in the isolators. In order to ensure efficient establishment of the indigenous gut microflora, droppings from healthy conventional rats were scattered on the food during the first day after removal from the isolators. The animals were housed similarly in both environments, in wire-mesh rat cages without bedding. They all received a cubed diet (Spiller's Small Animal Diet, Dalgety Ltd, Pangbourne, Berks, U.K.) sterilized by gamma-radiation, and weekly supplements of vitamin E, K and B_{12}. Sterile drinking water (Smedley's, Whiteleaf, Surrey) was supplied to the germ-free rats and tap water to their conventional controls.

The rats were reared to 8 weeks of age, during which time growth and physical condition were normal in both environments. Sterility checks, described by Fuller[7], were performed on the germ-free rats, which remained free from contamination throughout the experiment. At the end of the 8-week period the mean body weights of the 2 groups

were not significantly different (335 ± 27 g and 319 ± 31 g for the conventional and germ-free groups respectively). The animals were killed by guillotine; brain and liver were quickly removed and dropped into liquid nitrogen and thereafter stored at −20 °C until assay. Schwartz et al.[8] found no loss in rat brain MAO activity after storage at −80 °C for 1 year and we have found no loss in human platelet MAO activity after storage as a pellet for 1 year (unpublished data). Samples were thawed at room temperature (20 °C). These organs were homogenized in 50 vol. 0.1 M potassium phosphate buffer (pH 7.4). When phenylethylamine (PEA) was used as substrate, the liver homogenate was further diluted in 5 vol. of buffer and the brain homogenate, in 3 vol. MAO A activity was measured using ^{14}C-5-hydroxytryptamine (^{14}C-5-HT) (0.373 mM, 2.40 μCi/μmole and MAO B with ^{14}C-PEA (0.025 mM, 10.04 μCi/μmole) as substrates. Enzyme concentration was such that less than 5% of substrate was consumed. Both buffer and boiled enzyme blanks were used. 20 μl of homogenate or blank was incubated with 20 μl of substrate in 100 μl of buffer for 30 min at 37 °C in a shaking water bath and the reaction stopped with 100 μl of 2 M citrate. The products were extracted into toluene-ethyl acetate (1:1) 2.6 ml), when 5-HT oxidizing activity was measured or 2.6 ml toluene alone when PEA was substrate, shaking for 4 min and centrifuging at 800 × g for 2 min. Instagel (3 ml) was added and radioactivity counted in a liquid scintillation counter. Radiolabeled substrates were obtained from the Radiochemical Centre, Amersham, Bucks., U.K., and other chemicals from Sigma, London, U.K. Organic solvents were Analar grade (BDH Chemicals, London, U.K.). Protein was estimated according to the method of Lowry et al.[9] using bovine serum albumin as standard. All assays were carried out in duplicate. Statistical analysis was by Student's t-test (2-tailed).

Results. Mean values for body weight and protein content of homogenates between germ-free and conventional rats were not significantly different. However, both liver weight and brain weight were significantly greater in conventional rats. In the liver, 5-HT-oxidizing activity, but not PEA-oxidizing activity, was significantly greater in conventional compared with germ-free rats (table 1). However, there was no significant difference in enzyme activity, using either substrate, between brains of germ-free and conventional rats (table 2).

Discussion. The increased liver weight in conventional rats is a well established finding[5]. It is generally supposed that this change is the result of a greater work load because of the need to metabolize bacterial end-products as well as those of the host.

Our observation that 5-HT oxidation, presumably reflecting MAO A activity, is significantly higher in conventional compared with germ-free rat liver is quite different from the pattern observed in the chick[3,4] where both MAO A and B activity are augmented in the germ-free condition. Whether the difference is a manifestation of a selectively greater enzyme production in conventional rats or of the generation of selective MAO A inhibitor in germ-free animals, it did not extend to the brain. MAO is a flavoprotein[10]. Whilst certain vitamin B complex deficiencies have been reported in the germ-free state, e.g. of thiamine[11], no record of riboflavin status has so far appeared in the literature.

Differences in gut anatomy may contribute to the discrepancy between hepatic MAO activity in germ-free rat and chick. The chick has 2 caeca, just proximal to a very short colon. Unlike that of the rat, chick caeca do not become enlarged in the germ-free state and their contents continue to be ejected from time to time by strong peristaltic movements. The caecum becomes grossly enlarged in germ-free rats, perhaps because stasis produces an accumulation of compounds that affect muscle tone, vasodilation and other physiological properties[12]. It leads into a long colon so that, in the conventional animal, there is ample opportunity for absorption of bacterial metabolites that may be produced there. They may well include MAO substrates.

It has been known for many years that the gut flora is able to decarboxylate aromatic amino acids[13]; conversely, the germ-free state leads to amino acid accumulation in the caecal contents[14,15]. Significant amounts of monoamine substrates of MAO may well be generated therefore. It is of interest, in this context, to note that L-dopa administration in the rat, leading to dopamine excess, can result in an adaptive increase of MAO[16], and perhaps of MAO A alone[17]. Thus either more MAO A substrate is generated or MAO A is more easily inducible than MAO B and one of these possibilities accounts for the differences we have observed. Whatever the mechanism, it seems unlikely that a 14% increase, even though significant, can be of physiological significance for MAO is likely to be present in great excess compared with its substrates[18].

There is evidence that pharmacologically-active substances (e.g. 'alpha' pigment[19]) are elaborated in the caecum of germ-free rodents although their precise chemical nature has not been elucidated[8]. Their effects, which include decreased cardiac output and refractoriness to vasoactive substances such as adrenaline and vasopressin, are not apparent in the chick, either because these compounds are not produced or are not absorbed to the same extent. If a compound(s) inhibitory to MAO were similarly produced, it seems likely that its effect would be more marked in the rat than the chick.

Table 1. MAO activity (nmole substrate oxidized/mg protein/30 min) in rat liver

	No.	Body weight (g)*	Liver weight (g)*	5-HT-oxidizing activity*	PEA-oxidizing activity*
Conventional	12	335 ± 27	12.9 ± 1.2	106.7 ± 8.9	47.2 ± 7.1
Germ-free	12	319 ± 31	10.4 ± 1.2**	91.6 ± 7.1**	45.7 ± 3.5

* Mean ± SD. ** $p < 0.001$.

Table 2. MAO activity (nmole substrate oxidized/mg protein/30 min) in rat brain

	No.	Brain weight (g)*	5-HT-oxidizing activity*	PEA-oxidizing activity*
Conventional	12	1.69 ± 0.08	27.9 ± 3.0	14.8 ± 2.2
Germ-free	12	1.58 ± 0.07**	31.0 ± 4.5	15.2 ± 1.8

* Mean ± SD. ** $p < 0.001$.

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3 Phillips, A.W., Newcomb, H.R., Rupp, F.A., and Lachapelle, R., J. Nutr. *76* (1962) 119.
4 Böhm, K.H., Glover, V., Sandler, M., and Coates, M.E., Biochem. Pharmac. *28* (1979) 3345.
5 Gordon, H.A., and Pesti, L., Bact. Rev. *35* (1971) 390.
6 Gustafsson, B.E., Ann. N.Y. Acad. Sci. *78* (1959) 17.

7 Fuller, R., in: The germ-free animal in research, p.37. Ed. M.E. Coates. Academic Press, London 1968.
8 Schwartz, M.A., Aikens, A.M., and Wyatt, R.J., Psychopharmacologia *38* (1974) 319.
9 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
10 Walker, W.W., Kearney, E.B., Seng, R.L., and Singer, T.P., Eur. J. Biochem. *24* (1971) 328.
11 Wostmann, B.S., Knight, Jr, P.L., Keeley, L.L., and Kan, D.F., Fedn Proc. *22* (1963) 120.
12 Strandberg, K., Sedvall, G., Midvedt, T., and Gustafsson, B., Proc. Soc. exp. Biol. Med. *121* (1966) 699.
13 Gale, E.F., Adv. Enzymol. *6* (1946) 1.
14 Combe, E., Penot, E., Charlier, H., and Saquet, E., Annls Biol. anim. Biochim. Biophys. *5* (1965) 189.
15 Combe, E., and Saquet, E., C.r. Acad. Sci., Paris *262* (1966) 685.
16 Collins, G.G.S., Pryse-Davies, J., Sandler, M., and Southgate, J., Nature, Lond. *226* (1970) 642.
17 Mazumder, R.C., Glover, V., and Sandler, M., Biochem. Pharmac. *29* (1980) 1857.
18 Murphy, D.L., in: Monoamine oxidase and its inhibition, p.379. Eds G.E.W. Wolstenhome and J. Knight. Elsevier Excerpta Medica, Amsterdam 1976.
19 Gordon, H.A., and Kokas, E., Biochem. Pharmac. *17* (1968) 2333.

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Combined effect of ascorbic acid deficiency and underfeeding on the hepatic carnitine level in guinea-pigs

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Summary. Liver carnitine level decreased from 249±16.1 nmoles/g (mean ±SEM) control value to 148±9.8 nmoles/g (59.4%) in ascorbic acid deficient guinea-pigs, while in the underfed ('pair-fed') group it decreased to 181±14.1 nmoles/g (72.6%). Underfeeding also resulted in lower ascorbic acid levels; the depression of carnitine in the underfed animals could be prevented by an overdose (200 mg daily) of ascorbic acid.

According to the current view, carnitine (L-3-hydroxy-4-N-trimethylaminobutyrate) in combination with the related enzymes operates to transfer activated fatty acids from the cytoplasm into the mitochondrial matrix[1]. The pathway of carnitine biosynthesis has been well explored in animal[2,3] and human[4] studies. The proposed scheme involves 2 hydroxylating steps. The hydroxylase enzymes are dependent on α-ketoglutarate and ascorbic acid (AA)[2,5]. The stoichiometry and exact mode of action of AA, unlike those of α-ketoglutarate, were not defined by these in vitro studies[2,5].

In earlier studies of the in vivo effect of AA deprivation was found a severe depression of carnitine levels in guinea-pigs (unpublished). Later, it turned out that the reduced food ingestion, a concomitant symptom in the AA deficient state, can itself evoke carnitine depletion in both male and female animals. We reported depressed carnitine levels in the liver, serum and muscles of underfed male guinea-pigs[6]. The present work is devoted to revealing the relationship between AA deficiency and partial starvation in decreasing carnitine levels. For this purpose we determined the AA levels as well as those of carnitine in the livers of AA deficient and of underfed guinea-pigs supplied with normal amounts or an overdose of AA.

Methods. Young male guinea-pigs were used, with 350 g initial and 446 g terminal average body weights. Growth in itself did not alter the hepatic carnitine level, as was shown by separate measurements. An AA deficient diet with a low carnitine content (17.2 nmoles/g) was prepared as described previously[6]. This diet allowed the same growth as the commercial chow containing 50.2 nmoles/g carnitine[6]. The animals were fed this diet, and all of them were given 10 mg AA s.c. on every 2nd day for 2 weeks before starting the experiment. Then they were divided into 6 groups, as shown in the table. Average food consumption of the ad libitum fed AA-receiving animals (groups 1 and 3) was 30–40 g a day, 224 g in the 1st week and 266 g in the 2nd week of the experiment. The food supply for the underfed animals (groups 2 and 4) was limited to what was consumed by the AA-deficient animals (groups 5 and 6). In other words, groups 2 and 4 were 'pair-fed'. Quantitatively, the average consumption of the animals in groups 2, 4, 5, 6 gradually decreased (or was limited) to 10 g/day, so that they consumed 200 g in the 1st week and 115 g in the 2nd. An overdose of AA was supplied for groups 3 and 4 partly s.c. (100 mg/day) and partly per os (about 100 mg/day) with their drinking-water. On the 15hh day of the experiment the animals were decapitated between 08.00 and

Carnitine and ascorbic acid (AA) levels in the liver of AA deficient and underfed guinea-pigs

Group number and No. of animals	Terminal b.wt (g)	AA supply (daily mg)	Feeding	Carnitine in 1 g wet liver (nmoles)	AA in 1 g wet liver (μg)
1, n=6	446±12.2	5, s.c.	Free**	249±16.1	146±14.1
2, n=6	347±5.6	5, s.c.	Limited	181±14.1[a]	89±9.24[a]
3, n=6	437±16.1	100, s.c. 100, per os	Free**	269±20.1	213±17.2
4, n=6	349±16.7	100, s.c. 100, per os	Limited	255±21.4	167±11.2[d]
5, n=6	302±20.1	No	Free**	148±9.8[b]	Traces
6*, n=5	350±18.1	No	Free**	195±10.2[c]	Traces

Animals (initially weighing 352±11.8 g) were fed an AA deficient diet under the conditions indicated until the 15th day, when they were sacrified. *Animals in group 6 were sacrified on the 8th day. Values are means±SEM. **Free access to food. Levels of significance: [a]$p<0.01$; [b]$p<0.001$; [c]$p<0.05$; all vs group 1; [d]$p<0.05$ vs group 3.