Further evidence that the tick can transmit Saint Louis encephalitis was obtained in experiments with full-grown ticks and adult mice, since such adult mice upon which infected full-grown ticks had fed developed considerable resistance to intracerebral inoculation of virus. Full-grown ticks, which had engorged for a period of 5 to 7 days on infected adult mice, were transferred to normal adults and continued feeding for a like period. None of the latter developed convulsions, and all survived, although they seemed ill for several days showing lethargy, ruffled fur, and lack of interest in food. Later these convalescent mice were tested by intracerebral injection of Saint Louis encephalitis virus which was sufficient to kill, without exception, all normal controls in 3 to 5 days. Twelve of 17 mice so tested have survived, showing an apparently increased resistance to intracerebral inoculation. Here the possibility of infection by ingestion or by the intranasal route was obviated since adult ticks were protected during attachment by a specially contrived jacket having a small glass perforated chamber into which the tick may expand during engorgement.

Summary. 1. The virus of Saint Louis encephalitis was recovered from the bodies of engorged larval ticks which had fed on infected adult Swiss mice. 2. Under experimental conditions the Saint Louis encephalitis virus was transmitted to young Swiss mice by larval ticks. 3. Normal adult mice upon which infected, full-grown ticks had fed developed considerable resistance to intracerebral inoculation of virus.

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Vitamin A Concentration in Rat Liver During Recovery from CCl_4 Cirrhosis.*

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It has been found that the liver contains approximately 95% of the body stores of vitamin A,[1] and is the site of production of the

* Reported in preliminary form to the American Society of Zoölogists, *Anat. Rec.*, 1941, **81**, Suppl., 124.

1 Moore, T., *Biochem. J., London*, 1931, **25**, 275.

vitamin from its carotenoid precursors.[2] The vitamin A content of this organ is therefore a valuable index of vitamin A metabolism. Low levels of the vitamin in the blood[3–6] as well as in the liver[6–10] have been reported in human cirrhosis of the liver. Since the liver vitamin A may be decreased in amount by reduced vitamin A intake[11, 12] or by faulty gastrointestinal absorption,[9, 13] particularly as affected by the presence of jaundice,[14] studies were conducted in the rat in which an attempt was made to control these factors.

The data reported were obtained from rats in the recovery stage of carbon tetrachloride cirrhosis. At this stage the liver cells appear normal and the degree of fibrosis is moderate.[15] During the period of study the animals were free of jaundice and of diarrhea.

The effect of feeding different quantities of brewers' yeast on the concentration of vitamin A in cirrhotic livers was also examined.

Methods. Twenty-five 10-week-old, male rats, of Sherman stock, were employed. Nineteen of these were injected subcutaneously, twice weekly, with a 50% solution of CCl_4 in mineral oil, in a dosage of 0.05 ml per 100 g of body weight. The 6 animals receiving no CCl_4 served as controls.

The diet employed consisted of sucrose 69%, casein* 18%, butter 8%, Hawk-Oser salt mixture[16] 4%, supplemented by 0.3-2% of brewers' yeast.† The animals were fed *ad lib.* The CCl_4 injections were administered for 8 weeks, a period found sufficient to produce severe cirrhosis,[15] after which time the injections were discontinued.

2 Wilson, H. E. C., Ahmad, B., and Mazumdar, B. N., *Indian J. Med. Res.*, 1937, **25**, 85.

3 Lasch, F., *Klin. Wochenschr.*, 1938, **17**, 1107.

4 Lindqvist, T., *Acta med. Scand.*, 1938, **98**, Suppl. 97, pp. xii + 262 + 52.

5 Rubegni, R., *Policlinico, sez. med.*, Rome, 1939, **46**, 565.

6 Haig, C., and Patek, A. J., Jr., to be published.

7 Moore, T., *Biochem. J.*, London, 1937, **31**, 155.

8 Breusch, F., and Scalabrino, R., *Z. ges. exp. Med.*, 1934, **94**, 569.

9 Woo, T. T., and Chu, F. T., *Chinese J. Physiol.*, 1940, **15**, 83.

10 Ralli, E. P., Papper, E., Paley, K., and Bauman, E., *Arch. Int. Med.*, 1941, **68**, 102.

11 McCoord, A. B., and Luce-Clausen, E. M., *J. Nut.*, 1934, **7**, 557.

12 Lewis, J. M., Bodansky, O., Falk, K. G., and McGuire, G., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 248.

13 Crimm, P. D., and Short, D. M., *Ann. Int. Med.*, 1939, **13**, 61.

14 Breese, B. B., and McCoord, A. B., *J. Ped.*, 1940, **16**, 139.

15 Post, J., Earle, D. P., Jr., Patek, A. J., Jr., and Victor, J., *Am. J. Path.*, in press.

* Grade 20, Casein Company of America.

16 Hawk, P. B., and Oser, B. L., *Science*, 1931, **74**, 369.

† Harris Medicinal Brewers' Yeast, Tuckahoe, N.Y.

The 19 presumably cirrhotic rats[15] and the 6 normal, uninjected rats were then grouped as follows:

Group A: 10 "cirrhotic" rats received the basal ration supplemented by an average of 1% of brewers' yeast.

Group B: 9 "cirrhotic" rats received the basal ration supplemented by an average of 7% of brewers' yeast.

Group C: 6 normal rats received the basal ration supplemented by an average of 1% of brewers' yeast.

The source of vitamin A in the basal diet was butter. It was estimated[17] that this supplied 186-230 international units of vitamin A per kilo of body weight per day. This amount is 10 times the minimal requirement of vitamin A for growth as determined by Goss and Guilbert.[18] All diets were made at the same time and kept under identical environmental conditions.

Group A animals were fed *ad lib.* (12.5 g/day). Animals of group B were fed the same amount of food (12.9 g/day) as those of group A consumed. Group C animals, although fed *ad lib.*, consumed the same amount of food (13.3 g/day) as those of groups A and B. Thus the 3 groups of animals were maintained under isocaloric conditions and their vitamin A intake was the same.

The animals were kept on these rations for an additional 3 months, *during which time no more CCl_4 was administered.* All animals were then sacrificed and the body weight, liver weight, degree of hepatic fibrosis,[15] and amount of vitamin A per gram of liver tissue were determined.

The method employed for the preparation of vitamin A extracts of the livers was similar to that described by McCoord and Luce-Clausen.[11] Sampling errors were minimized by employing pooled samples taken from at least 6 different loci of each liver. The vitamin A contents of the liver extracts so prepared were determined by the method described elsewhere[19] for determining the vitamin A content of plasma extracts. The results were expressed in international units of vitamin A per gram of fresh liver tissue.

Results. In Table I are presented the body weights, liver weights, and concentrations of vitamin A in the livers of the 3 groups of animals under study. The average amount of vitamin A per gram of liver of the rats in the recovery stage of CCl_4 cirrhosis (groups A and B) is seen to be less than half that of the normal animals (group

17 Daniel, E. P., and Munsell, H. E., *U. S. Dept. Agric. Misc. Publ.*, No. 275, June, 1937.

18 Goss, J., and Guilbert, H. P., *J. Nut.*, 1939, **18**, 169.

19 Haig, C., and Patek, A. J., Jr., to be published.

TABLE I.
Body and Liver Weights in Grams, and International Units of Vitamin A per Gram of Rat Liver.

Group A (CCl_4, 1% yeast)				Group B (CCl_4, 7% yeast)				Group C (No CCl_4, 1% yeast)			
Rat No.	Body wt	Liver wt	Vit. A/g liver	Rat No.	Body wt	Liver wt	Vit. A/g liver	Rat No.	Body wt	Liver wt	Vit. A/g liver
10	156	3.7	**5.6**	3	208	5.8	**12.0**	53	172	12.7	**27.0**
16	205	6.2	**12.6**	7	278	8.5	**21.7**	54	250	8.1	**25.9**
21	168	4.6	**5.7**	9	304	9.0	**27.6**	56	211	5.3	**12.9**
22	167	5.5	**16.2**	17	286	9.5	**7.1**	57	216	5.7	**43.7**
32	169	8.1	**7.1**	25	364	9.5	**8.5**	59	226	5.5	**27.5**
33	167	5.0	**30.7**	30	318	9.0	**3.9**	60	254	6.8	**24.4**
35	181	5.5	**9.2**	42	317	10.5	**0.0**				
41	226	7.0	**22.5**	51	250	5.5	**15.4**				
43	202	9.0	**5.0**	52	265	6.5	**22.6**				
45	237	7.5	**4.7**								
Mean			**11.9**				**13.2**				**26.9**
Standard deviation			**8.3**				**8.8**				**9.0**

C). Despite the high degree of dispersion of the vitamin A measurements, the observed differences between the mean values for the CCl_4 injected rats and the uninjected rats were found to have statistical significance.

Since changes in liver size accompanying the reduction in vitamin A concentration would alter the interpretation of these findings, it is pertinent that the average ratio of body weight to liver weight was found to be the same for the two groups of animals, *viz.*, 33.4 for the control and 33.6 for the injected rats. Hence it is probable that the CCl_4 injections had no effect upon the size of the livers of the injected animals. Correlatively, it was found that for the injected rats the average ratio of the total vitamin A contents of the livers divided by the body weights was less than half that for the control rats. Hence the reduced concentration of vitamin A in the livers of the CCl_4 injected animals involved a proportionate reduction in the quantity of vitamin A available to the body from the liver, as well as in the total quantity of vitamin A stored in the liver.

No apparent correlation was found between the vitamin A concentration, the degree of fibrosis observed in microscopic sections of the individual livers,[15] and the yeast intake of the animals. This finding with regard to yeast intake confirms in part the observations of Dann and Moore.[20]

The data of Greaves and Schmidt[21] suggest that the administration of phosphorous for 6 days causes some interference with the conversion of carotene to vitamin A in the liver. These authors also

[20] Dann, W. J., and Moore, T., *Biochem. J.*, London, 1931, **25**, 914.

[21] Greaves, J. D., and Schmidt, C. L. A., *Am. J. Physiol.*, 1935, **111**, 503.

state that the administration of $CHCl_3$, benzene, or CCl_4 (for an unspecified number of days) to vitamin A-deficient rats, in the "dosage found possible to administer," had no demonstrable effect on the carotene conversion when this was determined by the vaginal smear method.

Studies by Wendt and König[22] and Cox[23] were concerned with comparisons between the vitamin A content of different portions of the same diseased liver, but offer no data comparing these values with those found in normal livers. Our analysis compared the vitamin A content of diseased and normal livers. Other studies[22, 24] of the influence of liver damage on storage of vitamin A in the liver, unlike the present experiments, were concerned with poisoning of the liver for only a few days. Moreover, in none of these experiments was CCl_4 employed as the poisoning agent.

It is important to emphasize that the CCl_4-injected rats of the present study had remained untreated for 3 months before the vitamin A analyses were made. At this stage the degree of liver fibrosis was minimal and the liver cells appeared normal. Despite these facts the livers of the injected animals contained less than half the amount of vitamin A found in the livers of the normal controls.

Summary. Nineteen rats were rendered severely cirrhotic by the bi-weekly administration of carbon tetrachloride over a period of 2 months. Three months after discontinuance of the CCl_4 injections the hepatic cells in the livers of these rats appeared histologically normal and very little fibrosis was present. At this time the mean vitamin A concentration in the livers was found to be 12.5 ± 8.6 international units per gram of fresh tissue. The livers of 6 control rats receiving the same amount of food and of vitamin A as the CCl_4 treated rats had a mean vitamin A concentration of 26.9 ± 9 I.U./g. The difference between these values was found to have statistical significance.

No correlation was observed between the vitamin A concentration in the cirrhotic livers and (1) the liver weight, or (2) the degree of fibrosis, or (3) the brewers' yeast intake of the animals.

It is concluded that the livers of rats recovering from CCl_4 cirrhosis contain much less vitamin A than do the livers of normal rats on the same diet.

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22 Wendt, H., and König, D., *Klin. Wochenschr.*, 1937, **16**, 1253.

23 Cox, A. J., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 333.

24 Lasch, F., and Roller, D., *Klin. Wochenschr.*, 1936, **15**, 1636.