produced by the organisms. Chick assays and quantitative analyses by the method of Trenner and Bacher[7] developed for vitamin K_1 proved that the yield of vitamin K_2 from the autolyzed bacilli is not significantly altered when the nutrient is previously extracted with ether. Furthermore biological assays on the nutrient itself indicated that not more than 10% of the vitamin present in the final culture can be introduced from this source.

The synthesis of a substance with K activity during putrefaction of fish meal and rice bran was first pointed out by Almquist and Stokstad.[8] It was found later that species of bacteria grown on the usual media also produced an antihemorrhagic factor.[9] No attempt, however, was made by these investigators to isolate and characterize the active principle. The well-known isolation of K_2 from putrefied fish meal[5] and this report of its isolation from the autolyzed cellular material of a spore-forming soil bacillus suggest that vitamin K_2 is a bacterial metabolite. In this connection the observation of Woolley and McCarter[10] that antihemorrhagic compounds as vitamin K concentrate, 2-methyl-1, 4-naphthoquinone, and phthiocol stimulates the growth of Johne's bacillus (*Mycobacterium paratuberculosis*) is noteworthy.

Summary A substance has been isolated from the autolyzed cellular material of the spore forming soil organism *Bacillus brevis* which, by both chemical and biological examination, is identical with vitamin K_2.

7 Trenner and Bacher, *J. Biol. Chem.*, 1941, **137**, 745.

8 Almquist and Stokstad, *J. Nutrition*, 1936, **12**, 329.

9 Almquist, Pentler, and Meech, PROC. SOC. EXP. BIOL. AND MED. 1938, **38**, 336.

10 Woolley and McCarter, PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 357.

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Effect of Amino Acid Deficiency on Rat Liver Before and After Partial Hepatectomy.*

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Since the remaining portion of rat liver after partial hepatectomy regenerates to normal size within 10 to 14 days it seemed of interest to see what effect a dietary deficiency of some essential amino acids might have on the formation of nucleic acids during this period of extremely rapid growth.

Partial hepatectomy was performed on rats that had been fed diets whose protein content was exclusively casein, zein, or gelatin. The livers at the time of hepatectomy and 4 days later were analyzed for ribonucleic acid, desoxyribonucleic acid, tryptophane, percentage regeneration, total nitrogen and total solids. Values for ribonucleic acid (P.N.A.) and desoxyribonucleic acid (D.N.A.) were obtained by the method of Schmidt and Thannhauser.[1] The determination of tryptophane was made by the procedure of Horn and Jones.[2] The percentage regeneration of the liver after partial hepatectomy is the increase in weight of the remnant as percentage of the amount of tissue removed. Since, on

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1 Schmidt, Gerhardt, and Thannhauser, S. J., *J.B.C.*, 1945, **161**, 83.

2 Horn, M. J., and Jones, D. B., *J.B.C.*, 1945, **157**, 153.

the average, two-thirds of the liver is removed at partial hepatectomy,

$$\% \text{ Regeneration} = \frac{(\text{Wt of liver after regeneration} - \tfrac{1}{2}\text{wt of tissue removed}) \, 100}{\text{Wt of tissue removed}}$$

Total solids were determined by drying the tissue to constant weight at 104°C. Micro-Kjeldahl with direct nesslerization was used for the nitrogen determinations. Differences were considered statistically significant when the Fischer t value exceeded 3.0.

The basic control diet consisted of 25% casein, 50% sucrose, 20% Crisco, 5% salt mixture (Phillips and Hart[3]) and a vitamin supplement of 0.5 ml "Vi Penta Drops" (Roche) per kilo. In the test diets the casein was replaced by zein or gelatin. One group of rats was given their maintenance ration of "Purina Chow" during the experimental period. The rats were kept on the respective diets for 10 days, then partially hepatectomized by the removal of the left lateral and median lobes. The rats continued on their diets during the 96 hour period of regeneration following the hepatectomy and then were killed for the final analysis of the livers. The 96 hour period was chosen because it has been shown that the most active cellular proliferation occurs within 72 to 96 hours.[4,5]

Results. (1) *Pre-Hepatectomized Liver.* The total solids and both P.N.A. and D.N.A. were the same on the zein and gelatin diets as on casein and Purina, while the tryptophane, though lower, was not greatly so. The total nitrogen on gelatin was also somewhat lower, though the significance was questionable, but was markedly reduced on the zein as compared with the other diets. Kosterlitz and Campbell[6] observed that in rats on a diet of zein with added lysine or tryptophane there was a considerable loss of liver protein as measured by non-lipid, non-glycogen liver solids. In a later paper Kosterlitz[7] reported a similar finding with an 18% gelatin diet, but he observed no change in total nucleic acid concentration of the liver. However, when his results were calculated per 100 g initial body weight instead of per 100 g liver weight, decreases in protein, phospholipin and nucleic acid were found. From indirect evidence he suggests that only cytoplasmic P.N.A. and not nuclear D.N.A. is affected. Later, by direct determination, Campbell and Kosterlitz[8] found that the D.N.A. content of liver tissue of rats, calculated for 100 g of initial body weight was not influenced by the protein content of the diet while P.N.A. was lost as had been previously suggested.

(2) *Regenerating Liver.* The regenerating liver, after 96 hours, has, we find, the same content of total solids on all diets, and is unchanged from the pre-hepatectomy liver. The concentration of D.N.A. in the regenerating livers of rats on the casein and purina diets was the same as that of the organ before hepatectomy. The P.N.A. content, however, was significantly greater. This differs from the observations of Davidson and Waymouth[4] who found no significant difference between the nucleoprotein of livers before and after partial hepatectomy. This extra mobilization of P.N.A. was observed also on a gelatin diet, but not when zein was the sole source of protein. The D.N.A. on these two diets was not significantly altered after hepatectomy.

Our values for tryptophane in the regenerating livers of rats on all diets tended to be lower than that of liver before hepatectomy, but not significantly so. In regenerating liver, the tryptophane values were somewhat less on the zein and gelatin diets than on the control casein diets, yet the striking thing was that they were so nearly alike, in spite of the fact that tryptophane was lacking in 2 of the diets.

Brues, Drury, and Brues[9] reported that the nitrogen content of regenerating liver after partial hepatectomy rose from a low value of 2.67% on the first day, but was still below normal (3.51%) well into the second week.

[3] Phillips, P. H., and Hart, E. B., *J.B.C.*, 1935, **109**, 657.

[4] Davidson, J. N., and Waymouth, C., *Biochem. J.*, 1944, **38**, 385.

[5] Drabkin, D. L., *J.B.C.*, 1947, **171**, 395.

[6] Kosterlitz, H. W., and Campbell, Rosa M., *J. Physiol.*, 1945-46, **104**, 16P.

[7] Kosterlitz, H. W., *J. Physiol.*, 1947, **106**, 194.

[8] Campbell, R. M., and Kosterlitz, H. W., *J. Physiol.*, 1947, **106**, 12P.

TABLE I.
Analyses of Livers.

Diet	Total solids, %	Nucleic acid phosphorus mg/100 g wet wt P.N.A.	Nucleic acid phosphorus mg/100 g wet wt D.N.A.	Tryptophane, % wet wt	Total N, % wet wt	% Regeneration
		At hepatectomy.				
Purina	31 ± 0.1* (9)	99 ± 2.3 (25)	23 ± 1.1 (25)		3.45 ± 0.07 (9)	
Casein	29 ± 0.2 (18)	99 ± 2.8 (15)	23 ± 1.1 (20)	0.25 ± 0.01 (15)	3.37 ± 0.08 (13)	
Zein	30 ± 1.0 (26)	99 ± 1.7 (13)	23 ± 3.0 (13)	0.22 ± 0.01 (11)	2.49 ± 0.14 (11)	
Gelatin	28± 1.0 (21)	96 ± 1.7 (19)	27 ± 1.7 (19)	.021 ± 0.007 (19)	3.07 ± 0.08 (15)	
		After 96 hr regeneration.				
Purina	27 ± 0.5 (10)	111 ± 1.5 (10)	20 ± 2.2 (9)		2.97 ± 0.07 (10)	61 ± 6.7 (10)
Casein	30 ± 1.1 (15)	115 ± 3.4 (16)	25 ± 2.0 (16)	0.22 ± 0.005 (14)	3.17 ± 0.12 (11)	67 ± 3.5 (16)
Zein	29 ± 0.8 (14)	98 ± 2.2 (15)	19 ± 1.2 (14)	0.21 ± 0.012 (10)	2.36 ± 0.11 (10)	63 ± 2.6 (23)
Gelatin	28 ± 1.0 (15)	109 ± 3.0 (17)	20 ± 1.4 (17)	0.19 ± 0.007 (13)	2.94 ± 0.14 (11)	73 ± 4.5 (15)

* Standard deviation of the mean.
Figures in parentheses indicate the number of animals.

In our experiments, however, the nitrogen concentration of the regenerated liver of rats on the casein diet, though somewhat lower than before hepatectomy, was not significantly so except on a "Purina Chow" diet. On the zein diet (but not on gelatin) we did observe a marked lowering of the nitrogen concentration in regenerating liver as compared with the casein control, the t value for this difference being 5.0. This is in line with the findings of Harrison and Long[10] who reported that zein, gelatin and gliadin were inadequate for the restoration of liver protein following depletion by a 48 hour fast. They used liver nitrogen in mg per 100 g of body weight as a measure of cytoplasmic protein.

Conclusion. 1. The total weight and total solids of regenerated liver 96 hours after partial hepatectomy are unaffected by diets in which gelatin and zein are the sole sources of protein.

2. On a casein diet, the regenerated liver has a higher P.N.A. content than the pre-hepatectomy liver. This is also true on a gelatin diet and on a purina chow diet, but not on a zein diet, indicating that the apparent stimulus to extra P.N.A. content normally induced in the regenerating liver, is inhibited by the lack of dietary lysine. There is no significant effect on the D.N.A. content either before or after hepatectomy, on any of the diets.

3. On gelatin and zein diets, the tryptophane content of the pre-hepatectomy livers was only slightly less than on adequate diets; the same held true for the regenerating liver, indicating that the liver was capable of obtaining almost its normal content of needed tryptophane from other sources.

4. In contrast to the constancy of the total solids, the total nitrogen of both the pre-hepatectomy and regenerating liver was significantly less on the zein diet than on the Purina chow, casein, and gelatin diets. This implies that on the zein diet, the decreases in nitrogen were associated with an increase in other constituents. On the basis of Kosterlitz's (loc. cit.) results, this may have been glycogen.

We are indebted to Corn Products Refining Company for the supply of zein.

[9] Brues, A. M., Drury, D. D., and Brues, M., *Arch. Path.*, 1936, **22**, 658.

[10] Harrison, Helen C., and Long, C. N. H., *J.B.C.*, 1945, **161**, 545.