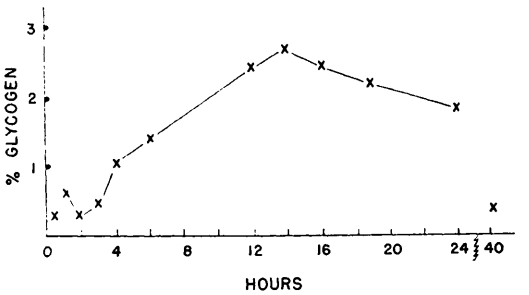

FIG. 1.

cine from the gastrointestinal tract of the white rat was found to be 102 g per 100 g of body weight per hour. The rate did not vary significantly during absorption periods of from 0.5 to 3 hours. Glycine is definitely glyconeogenic reaching a peak of glycogen production 14 hours after its administration. A smaller but definite peak was observed at one hour.

1. Wilson, R. H., and Lewis, H. B., *J. Biol. Chem.*, 1929, v84, 511.
2. Wilson, R. H., *J. Biol. Chem.*, 1929-30, v85, 559.
3. ———, *J. Biol. Chem.*, 1932, v97, 497.
4. Butts, J. S., Dunn, M. S., and Hollman, L. F., *J. Biol. Chem.*, 1935-36, v112, 263.
5. Stöhr, R., *Biochem. Z.*, 1938, v299, 242.
6. Mackay, E. M., Wick, A. N., and Carne, H. O, *J. Biol. Chem.*, 1940, v132, 613.
7. Olsen, N. S., Hemingway, A., and Nier, A. O., *J. Biol. Chem.*, 1943, v148, 611.
8. Alexander, B., Landwehr, G., and Seligman, A. M., *J. Biol. Chem.*, 1945, v160, 52.
9. Pope, C. G., and Stevens, M. P., *Biochem. J.*, 1939, v33, 1070.
10. Hess, W. C., and Shaffran, I. P., *J. Am. Chem. Soc.*, 1951, v73, 474.
11. Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, v100, 485.

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Effect of High Fat Diet on the Potassium Deficiency Syndrome in the Rat.* (19891)

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Potassium deficiency has been studied by a number of workers and physiological and pathological effects of the deficiency have been described in rats, mice, dogs, and calves(1). In a previous experiment it was observed(2) that when rats were fed potassium deficient diets containing 5% and 20% corn oil, the resulting deficiencies were more severe when the diet contained 20% corn oil. Since these diets were fed *ad libitum*, the observed results could have been due to decreased feed and potassium consumption by the rats receiving the 20% corn oil diet. The experiment herein described was designed to determine if this were the case, or if the higher level of fat in the diet increased the rat's need for potassium.

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TABLE I. Percentage Composition of Diets.

	Diet 1	Diet 2	Diet 3	Diet 4
Sucrose	39.2	73.2	39.2	73.2
Casein*	19.3	19.3	19.3	19.3
Corn oil	20	5	20	5
Na and K free salts	2.5	2.5	2.5	2.5
$NaHCO_3$	.91	.91	.91	.91
$KHCO_3$	.08	.08	.08	1.28
Vitamins†	.053	.053	.053	.053
Choline	.1	.1	.1	.1
Cellu-flour‡	19	—	19	—

* GBI-vit. free.
† Vitamins—mg/kg of diet: thiamine, 3; riboflavin, 3; pyridoxine, 2; Ca-pantothenate, 20; inositol, 100; niacin, 20; para-aminobenzoic acid, 250; biotin, .1; folic acid, .25.
‡ Chicago Dietetic Supply House, Chicago, Ill.

Experimental procedure. Six 40-50 g weanling male albino rats of the Sprague-Dawley strain were placed on each of the 4 diets given in Table I. The salt mixture employed was that used by Grunert and Phillips(3) with sodium and potassium added as the bicar-

TABLE II. Summary of Results.*

	Lot 1 (.03% K, 20% corn oil)	Lot 2 (.03% K, 5% corn oil)	Lot 3 (.03% K, 20% corn oil)	Lot 4 (.5% K, 5% corn oil)
Skeletal muscle				
meq. % Na	6.55 ± 1.3	5.22 ± 1.1	5.30 ± 2	1.94 ± .42
meq. % K	5.48 ± 1.7	6.40 ± .2	5.56 ± .8	9.35 ± .68
Kidney				
mg fresh wt/100 g body wt	1282 ±160	1274 ±67	1433 ±268	567 ±21
mg dry wt/100 g body wt	224 ± 25.6	242 ±24	239 ± 11	135 ±10
% water	82.5 ± .4	80.8 ± 1.7	83 ± 2.1	75.9 ± .6
Myocardial necrosis and fibrosis†	+ + + / + +	— + — / — — —	+ + +	— — — / — — —
Dilatation of renal tubules†	+ + + / + +	— + — / — — —	+ + +	— — — / — — —

* Stand. dev. are given.
† Each + indicates an animal in which the specified lesion was moderate or extensive.
Each — " " " " " " " " " slight or absent.

bonate when needed. Diets 1, 2, and 3 contained 0.25% sodium and 0.03% potassium and diet 4 contained 0.25% sodium and 0.5% potassium. Diets 1 and 3 were made isocaloric with diets 2 and 4 by the addition of cellu-flour as indicated. The rats which received diets 1 and 2 were pair-fed throughout the experimental period and diets 3 and 4 were fed *ad libitum*. Thus, the dietary intake of calories and of potassium for each of the rats receiving diet 2 was the same as that of its diet 1 pair mate. The original plan was to maintain the rats on the experimental diets for six weeks. Death of one diet 1 and three diet 3 rats on the twenty-fourth day of the experiment, however, prompted the termination of the experiment at that time. The deaths of these animals appeared to be due to obstruction of the large intestine by impaction of large masses of cellulose. The surviving animals were killed with natural gas, autopsied, and specimens were removed for sectioning and sodium, potassium, or water analyses. Heart and kidney sections were fixed in Bouin's fluid, embedded in paraffin, and stained routinely with hematoxylin and eosin for histological study. The left kidney of each rat was weighed to the nearest 0.2 mg on a Roller-Smith torsion balance and analyzed for moisture by drying to constant weight. Specimens of skeletal muscle were analyzed for sodium and potassium with a Perkin-Elmer Model 52A flame photometer after wet ashing.

Results and discussion. There were no statistically significant differences in the sodium or potassium concentrations in the striated muscle or in the per cent water in the kidneys of the rats on the 3 potassium deficient diets (Table II). There was a trend, however, toward more sodium and less potassium in the muscle sample, and increased water in the kidneys of the rats receiving diet 1 (.03% K, 20% corn oil) as compared with the values for the diet 2 (.03% K, 5% corn oil) animals. The animals on all 3 of the potassium deficient diets had greatly reduced potassium and increased sodium in their skeletal musculature and greater fresh and dry kidney weights per 100 g of body weight when compared with their respective controls. All of these effects of potassium deficiency except the increase in dry weight of kidney per unit of body weight are well known.

Examination of the heart and kidney sections revealed that the rats which had received diet 1 (.03% K, 20% corn oil) had much more extensive myocardial fibrosis and renal tubule dilatation than the rats which received diet 2 (.03% K, 5% corn oil). All of the surviving diet 1 rats had moderate to extensive myocardial fibrosis and kidney tubule dilatation, while only one of the diet 2

animals had myocardial or renal pathology that could be considered to be more than slight.

The reason for these results is not known. Rix and Ebrhardt(4) observed fluctuations in the potassium and calcium concentrations in the blood sera of rabbits when linseed oil was added to the rabbits' hay and turnip diet as daily 5 cc doses. They believed that the increase in serum potassium which they observed during the first 6 weeks of linseed oil administration was due to increased absorption of potassium from the intestine. They, however, were unable to explain the rapid fall in serum potassium concentration which occurred in their rabbits after the first 6 weeks on this regimen. The potassium content of the diet which they employed was not stated.

Summary. A diet containing 0.03% potassium and 20% corn oil produced a greater incidence and extent of the myocardial necrosis and fibrosis and dilatation of renal tubules typical of potassium deficiency than did an isocaloric, 5% corn oil diet containing the same amount of potassium when the 2 diets were pair fed. No statistically significant differences were observed in other criteria of potassium deficiency.

1. Follis, R. H., Jr., *The Pathology of Nutritional Diseases,* Chas. C. Thomas, Springfield, Ill., 1948.

2. Meyer, J. H., Perdue, H. S., and Phillips, P. H., unpublished.

3. Grunert, R. R., and Phillips, P. H., *J. Biol. Chem.,* 1949, v181, 821.

4. Rix, E., and Ebrhardt, L., *Z. Ges. Exptl. Med.,* 1936, v98, 748.

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Evidence for an Unidentified Growth Factor Required by *Microbacterium flavum.** (19892)

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Doetsch and Pelczar(1) reported that several strains of *Microbacterium lacticum* would grow in a medium consisting of vitamin-free acid-hydrolyzed casein, inorganic phosphates, calcium D-pantothenate and thiamin hydrochloride. Calcium D-pantothenate was shown to be essential for growth and thiamin to be stimulatory. The same workers(2) reported similar results when 18 amino acids were substituted for the casein hydrolysate. Supplementation of this latter medium with riboflavin, niacin, pyridoxine, biotin, folic acid, and glutamine produced growth comparable to that obtained in trypticase soy broth. *Microbacterium flavum,* however, was unable to grow in any of the synthetic media prepared. Bishop *et al.*(3) substantiated these results with *M. flavum* and found that addition of crude materials such as phytone, yeast extract, proteose-peptone No. 3, Wilson's Liver L, and asparagus juice resulted in media which supported good growth of *M. flavum.* The data indicated that a combination of substances was active in stimulating optimum growth. A further study of the nutritional requirements of this organism is the subject of this report.

Methods. Microbacterium flavum, OJ8, (ATCC 10340), was maintained on slants of Difco Micro Culture Assay Agar. Subcultures were made weekly, incubated for 24 hours at 30°C and then stored in the refrigerator. Inocula were prepared by transferring the stock cultures to 10 ml of Difco Micro Inoculum Broth and incubating at 30°C for 24 hours. The culture was centrifuged, washed

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